



US00910296B2

(12) **United States Patent**
Schroven et al.

(10) **Patent No.:** US 9,102,966 B2
(45) **Date of Patent:** Aug. 11, 2015

(54) **SYNTHESIS OF SIALOOLIGOSACCHARIDE DERIVATIVES**

(75) Inventors: **Andreas Schroven**, Barsel (DE); **Elise Champion**, Toulouse (FR); **Gyula Dekany**, Queensland (AU); **Christoph Röhrlig**, Mühlingen (DE); **Ioannis Vrasidas**, Thessaloniki (GR); **Ignacio Figueroa Pérez**, Miami, FL (US); **Markus Hederos**, Svedala (SE); **Julien Boutet**, La Plaine sur Mer (FR); **Ágnes Ágoston**, Telki (HU); **Piroska Kovács-Pénzes**, Jászberény (HU); **Ferenc Horváth**, Pilisszentkereszt (HU); **Christian Risinger**, Rottweil (DE); **Gergely Pipa**, Budapest (HU); **Sándor Demkó**, Debrecen (HU); **Lars Kröger**, Hamburg (DE)

(73) Assignee: **GLYCOM A/S**, KGS, Lyngby (DK)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 165 days.

(21) Appl. No.: **13/809,794**

(22) PCT Filed: **Jul. 15, 2011**

(86) PCT No.: **PCT/EP2011/062184**

§ 371 (c)(1),
(2), (4) Date: **Mar. 15, 2013**

(87) PCT Pub. No.: **WO2012/007588**

PCT Pub. Date: **Jan. 19, 2012**

(65) **Prior Publication Data**

US 2013/0171696 A1 Jul. 4, 2013

(30) **Foreign Application Priority Data**

Jul. 16, 2010 (GB) 1012036.8
May 13, 2011 (EP) 11166036

(51) **Int. Cl.**

C12N 9/00 (2006.01)
C12P 19/14 (2006.01)
C12P 19/44 (2006.01)
C12P 19/18 (2006.01)
C07H 1/06 (2006.01)
C07H 3/06 (2006.01)
C12P 19/26 (2006.01)

(52) **U.S. Cl.**

CPC . **C12P 19/14** (2013.01); **C07H 1/06** (2013.01);
C07H 3/06 (2013.01); **C12P 19/18** (2013.01);
C12P 19/26 (2013.01); **C12P 19/44** (2013.01)

(58) **Field of Classification Search**

CPC C07H 1/06; C07H 3/06; C12N 19/18;
C12N 19/26; C12N 19/28; C12N 19/44;
C12P 19/26; C12P 19/18; C12P 19/44

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2008/0306267 A1 * 12/2008 Rishel et al. 546/95

FOREIGN PATENT DOCUMENTS

WO	96/32492	10/1996
WO	2011/100979	8/2011
WO	2011/100980	8/2011
WO	2012/113404	8/2012
WO	2012/113405	8/2012
WO	2012/127410	9/2012
WO	2012/155916	11/2012
WO	2012/156897	11/2012
WO	2012/156898	11/2012

OTHER PUBLICATIONS

Cohen et al. *J. Org. Chem.* (2000) 65: 6145-6152.*

International Search Report mailed Oct. 31, 2011 in corresponding International Patent Application No. PCT/EP2011/062184.

Angela M. Scheppokat et al., "Enzymatic glycosylation, inhibitor design, and synthesis and formation of glyco-self assembled monolayers for simulation of recognition," *European Journal of Cell Biology*, vol. 89, pp. 39-52 (2010).

Deepani Indurugalla et al., "Natural sialoside analogues for the determination of enzymatic rate constants," *Org. Biomol. Chem.*, vol. 4, pp. 4453-4459 (2006).

Scott B. Cohen et al., "Synthesis and Characterization of an Anomeric Sulfur Analogue of CMP-Sialic Acid," *J. Org. Chem.*, vol. 65, No. 19, pp. 6145-6152 (2000).

Annie Malleron et al., "Chemoenzymatic synthesis of the 3-sulfated Lewis^a pentasaccharide," *Carbohydrate Research*, vol. 341, pp. 29-34 (2006).

Mahendra S. Sandbhor et al., "Substrate Recognition of the Membrane-Associated Sialidase NEU3 Requires a Hydrophobic Aglycone," *Biochemistry*, vol. 50, pp. 6753-6762 (2011).

Milady R. Ninonuevo et al., "A Strategy for Annotating the Human Milk Glycome," *J. Agric. Food Chem.*, vol. 54, No. 20, pp. 7471-7480 (2006).

Mitree M. Ponpipom et al., "Synthesis of Paragloboside Analogs," *Tetrahedron Letters*, No. 20, pp. 1717-1720 (1978).

Fengyang Yan et al., "Polymer-supported and chemoenzymatic synthesis of the *Neisseria meningitidis* pentasaccharide: a methodological comparison," *Carbohydrate Research*, vol. 328, pp. 3-16 (2000). Xian-wei Liu et al., "Characterization and synthetic application of a novel β 1,3-galactosyltransferase from *Escherichia coli* O55:1-17," *Bioorg. Med. Chem.*, vol. 17, pp. 4910-4915 (2009).

Anna Rencurosi et al., "Human milk oligosaccharides: an enzymatic protection step simplifies the synthesis of 3'- and 6'-*O*-sialyllectose and their analogues," *Carbohydrate Research*, vol. 337, pp. 473-483 (2002).

Joachim Thiem et al., "Chemoenzymatic Syntheses of Sialyloligosaccharides with Immobilized Sialidase," *Chem. Int. Ed. Engl.*, vol. 30, No. 11, pp. 1503-1505 (1991).

(Continued)

Primary Examiner — Susan Hanley

(74) *Attorney, Agent, or Firm* — Pillsbury Winthrop Shaw Pittman LLP

(57) **ABSTRACT**

The invention relates to a method for the synthesis of compounds of general formula (1A) and salts thereof wherein one of the R groups is an α -sialyl moiety and the other is H, X¹ represents a carbohydrate linker, A is a D-glucopyranosyl unit optionally substituted with fucosyl, R¹ is a protecting group that is removable by hydrogenolysis, the integer m is 0 or 1, by a transsialidation reaction.

(56)

References Cited**OTHER PUBLICATIONS**

- Rosalia Agusti et al., "Comparative rates of sialylation by recombinant trans-sialidase and inhibitor properties of synthetic oligosaccharides from *Trypanosoma cruzi* mucins-containing galactofuranose and galactopyranose," *Bioorg. Med. Chem.*, vol. pp. 2611-2616 (2007).
- Dirk Schmidt et al., "Sialidase-catalyzed transsialylation using polymer-supported solution-phase techniques," *Chem. Commun.*, pp. 1919-1920 (2000).
- Andre Lubineau et al., "Porcine liver (2→3)- α -sialyltransferase: substrate specificity studies and application of the immobilized enzyme to the synthesis of various sialylated oligosaccharide sequences," *Carbohydrate Research*, vol. 300, pp. 161-167 (1997).
- Dirk Schmidt et al., "Chemoenzymatic Synthesis of Sialyl Oligosaccharides with Sialidases Employing Transglycosylation Methodology," *J. Org. Chem.*, vol. 65, No. 25, pp. 8518-8526 (2000).
- Gastón Paris et al., "A Sialidase Mutant Displaying *trans*-Sialidase Activity," *J. Mol. Biol.*, vol. 345, pp. 923-934 (2005).

International Search Report mailed Mar. 26, 2012 in International Patent Application No. PCT/DK2012/050060 (International Publication No. WO 2012/113405).

International Search Report mailed May 10, 2011 in International Patent Application No. PCT/DK2011/050052 (International Publication No. WO 2011/100979).

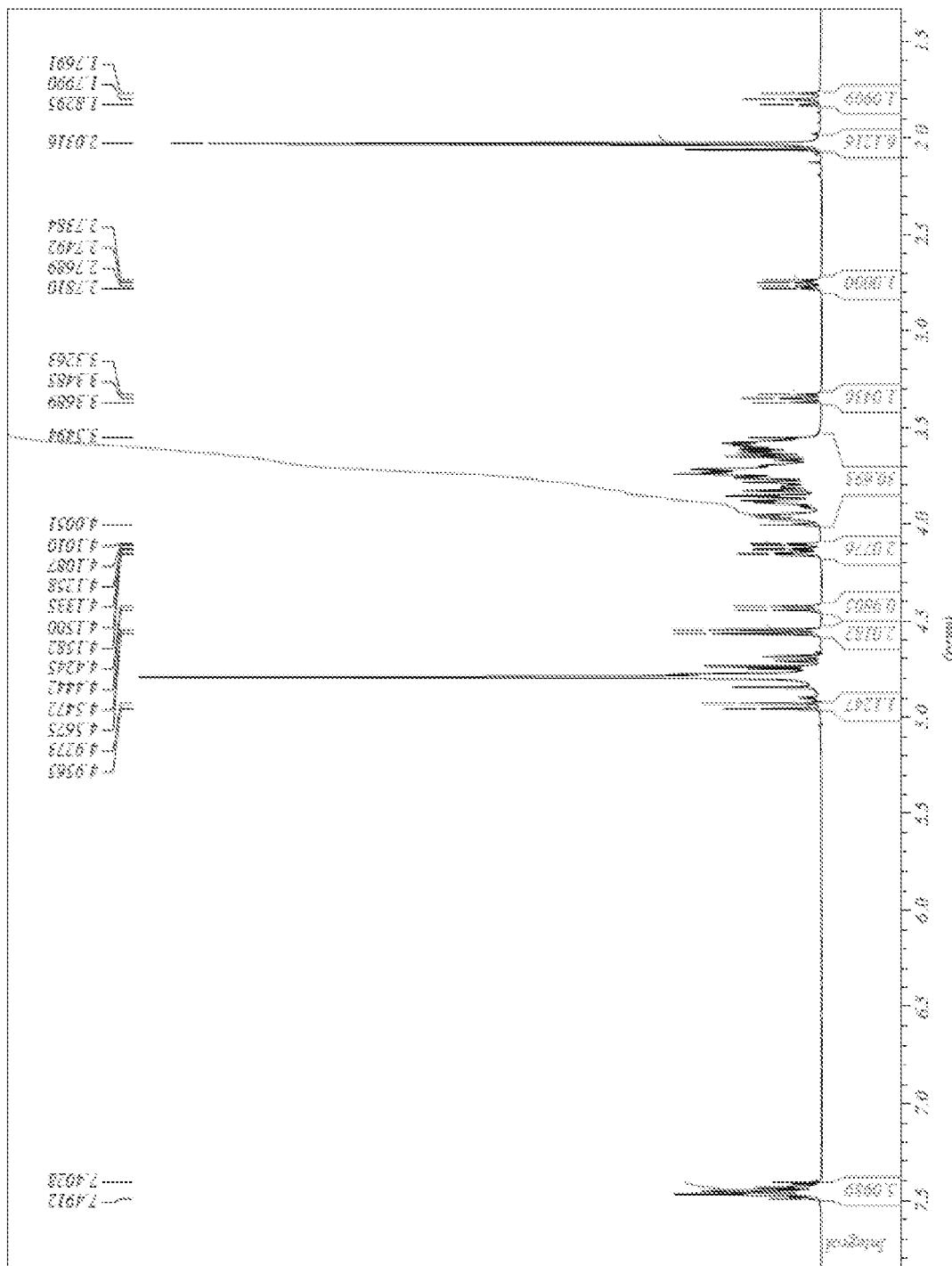
International Search Report mailed Jun. 13, 2012 in International Patent Application No. PCT/IB2012/051314 (International Publication No. WO 2012/127410).

International Search Report mailed Aug. 6, 2012 in International Patent Application No. PCT/DK2012/050170 (International Publication No. WO 2012/155916).

U.S. Office Action dated Nov. 19, 2014 in corresponding U.S. Appl. No. 13/809,829.

Green et al., "Protection for the Hydroxyl Group, Including 1,2- and 1,3-Diols," *Protective Groups in Organic Synthesis*, 2nd Edition, pp. 48-49 (1991).

* cited by examiner



1**SYNTHESIS OF SIALOOLIGOSACCHARIDE DERIVATIVES****CROSS REFERENCE TO RELATED APPLICATIONS**

This application is the National Phase entry of PCT/EP2011/062184, which claims priority to European Patent Application No. 11166036.1, filed May 13, 2011 and Great Britain Patent Application No. 1012036.8, filed Jul. 16, 2010. The content of these applications is incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to the enzymatic synthesis of sialooligosaccharide glycosides, and novel precursors and products taking part in the synthesis.

BACKGROUND OF THE INVENTION

Sialic acids are derivatives of the nine-carbon sugar neuraminic acid and encompass three parent molecules, N-acetyl-(Neu5Ac), N-glycolyl-(Neu5Gc) and deamino-neuraminic acid (3-deoxy-D-glycero-D-galacto-nonulosoic acid, KDN), which can be substituted at C-4, C-7, C-8 and C-9 by various moieties. They have many major biological roles, ranging from embryogenesis to neural plasticity to pathogen interactions. Although they may rarely occur in free form, they are usually found in chemical covalent linkage at the non-reducing terminus or in internal positions of oligosaccharide side-chains of glycoproteins and glycolipids. The linkages of sialic acids in which they are bound to penultimate sugars such as galactose, N-acetyl-galactosamine and N-acetyl-glucosamine are most commonly α -2,3- and α -2,6-ketosidic bonds.

are found to act as prebiotics in the human intestinal system helping to develop and maintain the intestinal flora. Furthermore they have also proved to be anti-inflammatory, and therefore these compounds are attractive components in the nutritional industry for the production of, for example, infant formulas, infant cereals, clinical infant nutritional products, toddler formulas, or as dietary supplements or health functional food for children, adults, elderly or lactating women, both as synthetically composed and naturally occurring compounds and salts thereof. Likewise, the compounds are also of interest in the medicinal industry for the production of therapeutics. In the human milk oligosaccharides the sialic acid residue is always linked to the terminal 3-O- and/or 6-O-position(s) of D-galactose via α -glycosidic linkage.

The availability of naturally occurring sialylated human milk oligosaccharides is limited. Mature human milk is the natural milk source that contains the highest concentrations of milk oligosaccharides (12-14 g/l), other milk sources are cow's milk (0.01 g/l), goat's milk and milk from other mammals. This low natural availability and difficult isolation methods are important motivations for the development of biotechnological and chemical methodologies for the production of these attractive compounds.

Approximately 200 HMOs have been detected from human milk by means of combination of techniques including microchip liquid chromatography mass spectrometry (HPLC Chip/MS) and matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry (MALDI-FT ICR MS) (Ninonuevo et al. *J. Agric. Food Chem.* 54, 7471 (2006)), from which to date at least 115 oligosaccharides have been structurally determined (Urashima et al.: *Milk Oligosaccharides*, Nova Medical Books, NY, 2011). These human milk oligosaccharides can be grouped into 13 core units (Table 1). About a quarter of oligosaccharides contains sialic acid.

TABLE 1

13 different core structures of human milk oligosaccharides (HMOs)		
No	Core name	Core structure
1	lactose (Lac)	Gal β 1-4Glc
2	lacto-N-tetraose (LNT)	Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc
3	lacto-N-neotetraose (LNnT)	Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc
4	lacto-N-hexaose (LNH)	Gal β 1-3GlcNAc β 1-3(Gal β 1-4GlcNAc β 1-6)Gal β 1-4Glc
5	lacto-N-neohexaose (LNnH)	Gal β 1-4GlcNAc β 1-3(Gal β 1-4GlcNAc β 1-6)Gal β 1-4Glc
6	para-lacto-N-hexaose (para-LNH)	Gal β 1-3GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc
7	para-lacto-N-neohexaose (para-LNnH)	Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc
8	lacto-N-octaose (LNO)	Gal β 1-3GlcNAc β 1-3(Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-6)Gal β 1-4Glc
9	lacto-N-neooctaose (LNnO)	Gal β 1-4GlcNAc β 1-3(Gal β 1-3GlcNAc β 1-3Gal β 1-4GlcNAc β 1-6)Gal β 1-4Glc
10	Iso-lacto-N-octaose (iso-LNO)	Gal β 1-3GlcNAc β 1-3(Gal β 1-3GlcNAc β 1-3Gal β 1-4GlcNAc β 1-6)Gal β 1-4Glc
11	para-lacto-N-octaose (para-LNO)	Gal β 1-3GlcNAc β 1-3 Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-6)Gal β 1-4Glc
12	Lacto-N-decaose (LND)	Gal β 1-3GlcNAc β 1-3[Gal β 1-4GlcNAc β 1-3(Gal β 1-4GlcNAc β 1-6)Gal β 1-4Glc]Gal β 1-4Glc
13	Lacto-N-neodecaose (LNnD)	Gal β 1-3GlcNAc β 1-3[Gal β 1-3GlcNAc β 1-3(Gal β 1-4GlcNAc β 1-6)Gal β 1-4Glc]Gal β 1-4Glc

Among sialoglycoconjugates, sialylated human milk oligosaccharides are of great importance which is directly linked to their unique biological activities such as antibacterial, antiviral, immune system and cognitive development enhancing activities. Sialylated human milk oligosaccharides

are found to act as prebiotics in the human intestinal system helping to develop and maintain the intestinal flora. Furthermore they have also proved to be anti-inflammatory, and therefore these compounds are attractive components in the nutritional industry for the production of, for example, infant formulas, infant cereals, clinical infant nutritional products, toddler formulas, or as dietary supplements or health functional food for children, adults, elderly or lactating women, both as synthetically composed and naturally occurring compounds and salts thereof. Likewise, the compounds are also of interest in the medicinal industry for the production of therapeutics. In the human milk oligosaccharides the sialic acid residue is always linked to the terminal 3-O- and/or 6-O-position(s) of D-galactose via α -glycosidic linkage.

gies have been developed for the isolation of some sialooligosaccharides from natural source.

The synthesis of complex sialooligosaccharides follows multistep synthetic pathways utilising protection and deprotection strategies. Stereoselective chemical synthetic processes can become very complicated due to the extensive use of protecting groups. These strategies give sialylated oligosaccharides via stereoselective O-sialylation of appropriate protected glycosyl acceptors using glycosylhalide, thioglycoside or diethylphosphite donor activations. The use of either very expensive or very toxic chemicals for the sialylation such as mercury cyanide, mercury bromide and silver carbonate is one of the reasons that make these methodologies less attractive. Inefficient stereocontrol and/or poor yields likewise make(s) the strategies less suitable for further developments. Additionally, these strategies are characterized by severe purification difficulties.

In the case of enzymatic production of sialooligosaccharides, sialyltransferases and sialidases have been the preferred enzymes used. These complex enzymatic systems represent very expensive methodologies for scale-up production and difficult purification protocols are likewise a hindrance for further technology developments. Sialidases could not be used successfully in large scale production methodologies due to low yields and lack of regio- and stereoselectivity. Although in some cases sialyltransferase enzymes are found to be effective in the synthesis of complex sialooligosaccharides (e.g. the synthesis of 1-O- β -benzyl glycoside of 3'-O-(N-acetyl-neuraminosyl)-lactose sodium salt: WO 96/32492; the synthesis of 1-O- β -(4,5-dimethoxy-2-nitro)-benzyl glycoside of 3'-O-(N-acetyl-neuraminosyl)-lactose sodium salt: Cohen et al. *J. Org. Chem.* 65, 6145 (2000)), the need of CMP-activated sialic acid (cytidine 5'-monophosphosialic acid) as sialyl donor—whose availability is, in fact, rather limited—for transferring the sialic acid portion to the acceptor oligosaccharide restricts their usefulness.

The ability of N-acetyl-lactosamine benzyl glycoside and benzyl glycosides of mucin oligosaccharides from *T. cruzi* to act as substrate in transsialidase reaction has been studied (Lubineau et al. *Carbohydr. Res.* 300, 161 (1997); Agusti et al. *Bioorg. Med. Chem.* 15, 2611 (2007)).

Regioselective sialidation of unprotected or anomERICALLY substituted galactose, lactose or N-acetyl-lactosamine derivatives by means of sialidases in poor yield has been reported (Thiem et al. *Angew. Chem. Int. Ed. Eng.* 30, 1503 (1991); Schmidt et al. *Chem. Comm.* 1919 (2000); Schmidt et al. *J. Org. Chem.* 65, 8518 (2000)).

Some biotechnological methodologies are also described using genetically modified bacteria, yeasts or other microorganisms. Such methods have serious drawbacks in regulatory processes due to limiting commercialisation opportunities.

Sialoglycoconjugates are known to be unstable under certain reaction conditions, such as to acid and base. Indeed, they are able to self-hydrolyse. Accordingly, conditions for preparation and purification of these compounds must be carefully selected.

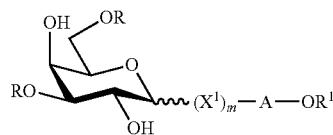
In summary, isolation technologies have never been able to provide large quantities of sialooligosaccharides due to the large number of oligosaccharides present in the pool of natural origin, e.g. in human milk. Additionally, the presence of regioisomers characterized by extremely similar structures further made separation technologies unsuccessful. Enzymatic methodologies suffer from the low availability of enzymes, extremely high sugar nucleotide donor prices and regulatory difficulties due to the use of enzymes produced in genetically modified organisms. The preparation of oligosaccharides via biotechnology has huge regulatory obstacles due

to the potential formation of several unnatural glycosylation products. Generally, all the chemical methods developed for the synthesis of sialooligosaccharides have several drawbacks which prevented the preparation of even multigram quantities of the target compounds (e.g. see the synthesis of 3'-O- and 6'-O-(N-acetyl-neuraminosyl)-lactose through the corresponding benzyl glycoside: Rencurosi et al. *Carbohydr. Res.* 337, 473 (2002)).

During the past decades the interest in the preparation and commercialisation of sialylated human milk oligosaccharides has been increasing steadily. There is still a need for novel methodologies which can simplify preparation and overcome or avoid purification problems encountered in prior art methods.

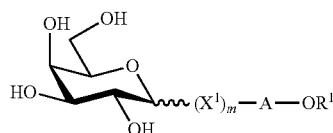
SUMMARY OF THE INVENTION

In one aspect, the present invention relates to a method for the synthesis of compounds of general formula 1A and salts thereof.



1A

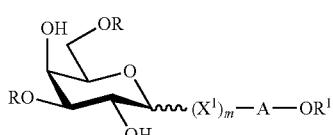
wherein one of the R groups is an α -sialyl moiety and the other is H, X^1 represents a carbohydrate linker, A is a D-glucopyranosyl unit optionally substituted with fucosyl, R^1 is a protecting group that is removable by hydrogenolysis, the integer m is 0 or 1, characterized in that a sialyl donor of formula SA-OR² or salts thereof, wherein R² can be a mono-, di- or oligosaccharide, glycolipid, glycoprotein or glycopeptide, cyclic or acyclic aliphatic group, or aryl residue, and SA is an α -sialyl moiety, is reacted with a sialyl acceptor of general formula 2A or a salt thereof



2A

wherein X^1 , A, m and R^1 are as defined above, under the catalysis of an enzyme having transsialidase activity.

In another aspect, the present invention provides compounds of general formula 1A' and salts thereof



1A'

wherein one of the R groups is an α -sialyl moiety and the other is H, R^1 is a protecting group that is removable by hydrogenolysis, A is a D-glucopyranosyl unit optionally substituted with fucosyl, integer m is 0 or 1, and X' represents a carbohydrate linker, provided that 1-O- β -benzyl and 1-O- β -

5

(4,5-dimethoxy-2-nitro)-benzyl glycosides of 3'-O—(N-acetyl-neuraminosyl)-lactose sodium salt, and 1-O- β -benzyl glycoside of 6'-O—(N-acetyl-neuraminosyl)-lactose sodium salt are excluded.

BRIEF DESCRIPTION OF THE FIGURES

The invention will be described in further detail hereinafter with reference to:

FIG. 1 which shows the 400 MHz ^1H -NMR spectrum of benzyl 3'''-O-sialyl- β -LNnT according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Whatever route is taken to synthesise an oligosaccharide, the final target unprotected oligosaccharide is soluble only in water, which presents challenges for the later steps of the synthesis. Organic solvents commonly used in synthetic manufacturing processes are not suitable for the reactions of the very final stages of the oligosaccharide synthesis.

The present invention provides new sialooligosaccharides protected in the anomeric position and methodology suitable for manufacturing thereof. The invention is based upon the utilisation of water soluble 1-O-protected oligosaccharide intermediates in transsialidation reaction, wherein the 1-O-protecting group chosen may be removed by hydrogenolysis. Preferably, the 1-O-protecting group should also provide to the oligosaccharide intermediate physical and chemical properties assisting powerful purification processes. For example, the introduction of an aromatic group such as a benzyl or substituted benzyl group as a hydrophobic moiety enables the derivatives to be soluble in organic protic solvents like alcohols while their water solubility also remains. This opens the possibility of using mobile phases having a wide range of water/alcohol proportions which can be applied in separation/purification techniques such as size exclusion or reverse phase chromatography. Moreover, with careful design of substituents on the aromatic group, crystalline compounds can in some cases be realized, which allows the development of powerful manufacturing procedures using crystallisation alone for product purifications. Furthermore, the benzylic 1-O-protecting group can be removed by catalytic reduction (hydrogenolysis) in the last step under mild and delicate conditions that prevent by-product formation, which is undoubtedly an advantage when at least one sialyl group is present in the target oligosaccharide. It is possible for the catalytic reduction to take place in aqueous solution.

General Terms

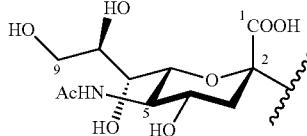
Throughout the present application the term “ α -sialyl moiety” or “sialyl moiety” present in the sialyl donors and in the compounds of general formula 1, refers to glycosyl moieties of any naturally occurring or modified neuraminic or sialic acid derivatives and analogues thereof having an α -glycosidic linkage, as depicted by the example of N-acetyl neuraminic acid in Scheme 1. Preferred neuraminic acids are N-acetyl-(Neu5Ac), N-glycolyl-(Neu5Gc) and deamino-neuraminic acid (3-deoxy-D-glycero-D-galacto-nonuliosonic acid, KDN). Also included are Neu5Ac, Neu5Gc and KDN derivatives that are derivatized with linkers, reactive functional groups, detectable labels or targeting moieties, and/or substituted at C-4, C-7, C-8 and/or C-9, especially at C-9, with acyloxy, alkoxy, halogen or azido. More preferred O-substituents are acetyl (at C-4, C-7, C-8 and/or C-9), lactyl (at C-9), methyl (at C-8), sulphate (at C-8) or phosphate (at

6

C-8). The preferred substituents on the amino group are acyls including glycolyl and acetoacetyl as well.

5

Scheme 1



The “protecting group that is removable by hydrogenolysis” refers to groups whose C—O bond to the 1-oxygen is cleaved by addition of hydrogen in the presence of catalytic amounts of palladium, Raney nickel or another appropriate metal catalyst known for use in hydrogenolysis, resulting in the regeneration of the OH group. Such protecting groups are well known to the skilled man and are discussed in *Protective Groups in Organic Synthesis*, P G M Wuts and T W Greene, John Wiley & Sons 2007. Suitable protecting groups include benzyl, diphenylmethyl (benzhydryl), 1-naphthylmethyl, 2-naphthylmethyl or triphenylmethyl (trityl) groups, each of which may be optionally substituted by one or more groups selected from: alkyl, alkoxy, phenyl, amino, acylamino, alkylamino, dialkylamino, nitro, carboxyl, alkoxy carbonyl, carbamoyl, N-alkylcarbamoyl, N,N-dialkylcarbamoyl, azido, halogenalkyl or halogen. Preferably, such substitution, if present, is on the aromatic ring(s). Particularly preferred protecting groups are benzyl or 2-naphthylmethyl groups optionally substituted with one or more groups selected from phenyl, alkyl or halogen. More preferably, the protecting group is selected from unsubstituted benzyl, unsubstituted 2-naphthylmethyl, 4-chlorobenzyl, 3-phenylbenzyl and 4-methylbenzyl. These particularly preferred and more preferable protecting groups have the advantage that the by-products of the hydrogenolysis are exclusively toluene, 2-methylnaphthalene, or substituted toluene or 2-methylnaphthalene derivatives, respectively. Such by-products can easily be removed even in multi ton scales from water soluble oligosaccharide products via evaporation and/or extraction processes.

Throughout the present description, the term “alkyl” means a linear or branched chain saturated hydrocarbon group with 1-6 carbon atoms, such as methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, s-butyl, t-butyl, n-hexyl, etc.

The term “aryl” refers to a homoaromatic group such as phenyl or naphthyl.

In the present description, the term “acyl” represents an R'—C(=O)—group, wherein R' may be H, alkyl (see above) or aryl (see above), such as formyl, acetyl, propionyl, butyryl, pivaloyl, benzoyl, etc. The alkyl or aryl residue may either be unsubstituted or may be substituted with one or more groups selected from alkyl (only for aryl residues), halogen, nitro, aryl, alkoxy, amino, alkylamino, dialkylamino, carboxyl, alkoxy carbonyl, carbamoyl, N-alkylcarbamoyl, N,N-dialkylcarbamoyl, azido, halogenalkyl or hydroxyalkyl, giving rise to acyl groups such as chloroacetyl, trichloroacetyl, 4-chlorobenzoyl, 4-nitrobenzoyl, 4-phenylbenzoyl, 4-benzamido-benzoyl, 4-(phenylcarbamoyl)-benzoyl, glycolyl, acetoacetyl, etc.

The term “alkyloxy” or “alkoxy” means an alkyl group (see above) attached to the parent molecular moiety through an oxygen atom, such as methoxy, ethoxy, t-butoxy, etc.

“Halogen” means fluoro, chloro, bromo or iodo.

“Amino” refers to a —NH₂ group.

"Alkylamino" means an alkyl group (see above) attached to the parent molecular moiety through an —NH-group, such as methylamino, ethylamino, etc.

"Dialkylamino" means two alkyl groups (see above), either identical or different ones, attached to the parent molecular moiety through a nitrogen atom, such as dimethylamino, diethylamino, etc.

"Acylamino" refers to an acyl group (see above) attached to the parent molecular moiety through an —NH-group, such as 10 acetylamino (acetamido), benzoylamino (benzamido), etc.

"Carboxyl" denotes an —COOH group.

"Alkyloxycarbonyl" means an alkyloxy group (see above) attached to the parent molecular moiety through a —C(=O)-group, such as methoxycarbonyl, t-butoxycarbonyl, etc. 15

"Carbamoyl" is an $\text{H}_2\text{N}-\text{C}(=\text{O})$ -group.

"N-Alkylcarbamoyl" means an alkyl group (see above) attached to the parent molecular moiety through a —HN—C(=O)-group, such as N-methylcarbamoyl, etc. 20

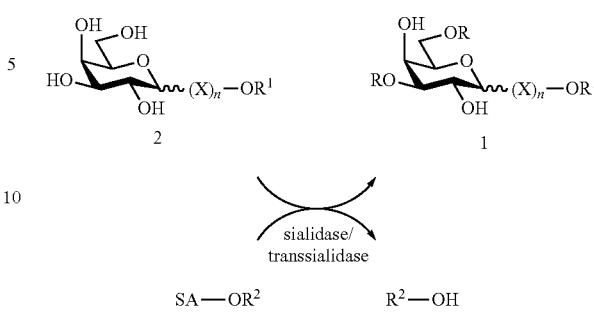
"N,N-Dialkylcarbamoyl" means two alkyl groups (see above), either identical or different ones, attached to the parent molecular moiety through a >N—C(=O)-group, such as 25 N,N-methylcarbamoyl, etc.

In the present description the term "salt" in connection with compounds of general formulae 1 and 2 and of formula SA-OR², which contain at least one sialyl residue, means an associated ion pair consisting of the negatively charged acid residue and one or more cations in any stoichiometric proportion. Cations, as used in the present context are atoms or molecules with positive charge. The cation may be inorganic as well as organic cation. Preferred inorganic cations are ammonium ion, alkali metal, alkali earth metal and transition metal ions, more preferably Na⁺, K⁺, Ca²⁺, Mg²⁺, Ba²⁺, Fe²⁺, Zn²⁺, Mn²⁺ and Cu²⁺, most preferably K⁺, Ca²⁺, Mg²⁺, Ba²⁺, Fe²⁺ and Zn²⁺. Basic organic compounds in positively charged form may be relevant organic cations. Such preferred positively charged counterparts are diethyl amine, triethyl amine, diisopropyl ethyl amine, ethanolamine, diethanolamine, triethanolamine, imidazol, piperidine, piperazine, morpholin, benzyl amine, ethylene diamine, meglumin, pyrrolidine, choline, tris-(hydroxymethyl)-methyl amine, N-(2-hydroxyethyl)-pyrrolidine, N-(2-hydroxyethyl)-piperidine, N-(2-hydroxyethyl)-piperazine, N-(2-hydroxyethyl)-morpholine, L-arginine, L-lysine, oligopeptides having L-arginine or L-lysine unit or oligopeptides having free amino group on N-terminal, etc., all in protonated form. Such salt formations can be used to modify characteristics of the complex molecule as a whole, such as stability, compatibility to excipients, solubility and ability to form crystals.

Transsialidation Reactions

In accordance with the present invention there is provided a process for synthesizing sialooligosaccharides of general formula 1 and salts thereof wherein one of the R groups is an α -sialyl moiety and the other is H, X represents a carbohydrate linker, R¹ is a protecting group that is removable by hydrogenolysis and the integer n is 0 or 1, characterized in that a sialyl donor of formula SA-OR² and salts thereof, wherein R² can be a mono-, di- or oligosaccharide, glycolipid, glycoprotein or glycopeptide, cyclic or acyclic aliphatic group, or aryl residue, and SA is an α -sialyl moiety, is reacted with a sialyl acceptor of general formula 2 and salts thereof, under the catalysis of an enzyme having transsialidase activity. The process is depicted in Scheme 2.

Scheme 2



An advantage of providing compounds of general formula 1 is the more simple purification of the sialylated oligosaccharide 1-O-protected glycosides compared to the unglycosylated sialooligosaccharides. Since there is no formation of free sialic acid as a side product, and due to the different polarity of the reaction compounds, isolation of the products by reverse phase or size exclusion chromatography is possible. In the case of reverse phase chromatography when water is used, compounds of general formula 1 migrate much more slowly than the very polar compounds present in the reaction mixture, thus the polar compounds can be eluted smoothly. Compounds of general formula 1 can be then washed from the column with e.g. alcohol.

Enzymes Having Transsialidase Activity

Enzymes having transsialidase activity and suitable for the purpose of the method of making sialooligosaccharides claimed in the present application can be selected from sialidase and transsialidase enzymes.

Sialidases (EC 3.2.1.18), classified in the GH33 family, are retaining enzymes with the ability of hydrolyzing the α -linkage of the terminal sialic acid, mainly those bound to galactose with α -2-3 or α -2-6 linkage, of various sialoglycoconjugates. They are found particularly in diverse virus families and bacteria, and also in protozoa, some invertebrates and mammals. Some bacterial sialidases can be used to scavenge sialic acids from sialylated glycoprotein, glycolipids or other glycoconjugates for nutrients for bacterial cell growth.

Although sialidases are characterized by their hydrolytic activity, under appropriate reaction conditions they are able to catalyze the transfer of a sialic acid unit to an asialo acceptor by a transsialidation reaction giving rise to the formation of sialoglycoconjugates. Sialidases from pathogen bacteria or viruses such as *Bacteroides fragilis*, *Clostridium* species (e.g. *C. perfringens*), *Corynebacterium diphtherias*, *Haemophilus parasuis*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Streptococcus pneumoniae*, *Tannerella forsythia*, *Vibrio cholerae* or Newcastle disease virus and from non-pathogenic bacteria or viruses such as *Actinomyces viscosus*, *Arthrobacter* species or *Micromonospora viridifaciens* are capable of acting as a catalyst for a sialylation reaction due to their transsialidase activity with α -2-3 and/or α -2-6 selectivity. As to the regioselectivity, the ratio between the α -2-3- and α -2-6-linked products varies depending on the enzymes and/or the acceptors. For example sialidases from *A. ureafaciens*, *C. perfringens* and *V. cholerae* have good α -2-6 selectivity, whereas those from *S. typhimurium* and Newcastle disease virus have good to excellent preference for formation of the α -2-3 linkage.

Recently, sialidases from *Bifidobacterium* species like *Bifidobacterium bifidum* and *Bifidobacterium longum* subsp. *infantis* have been identified, cloned and characterized. These

sialidases can cleave and so recognize both α -2,3- and α -2,6-linked sialosides. Sialidases from *Bifidobacterium longum* subsp. *infantis* have a consistent preference for α -2,6-linkage whereas sialidases from *Bifidobacterium bifidum* have a consistent preference for α -2,3-linkage.

In order to improve regioselectivity and/or conversion of the transsialidation reaction the sialidases may be subjected to alteration by various engineering techniques.

In rational engineering novel altered enzymes (mutants) are created by point mutation. The mutation generally affects the active site of the enzyme. Replacement of the catalytic nucleophile with a non-nucleophilic residue results in the formation of an inactive mutant or an altered enzyme with reduced transglycosylation activity due the lack of an appropriate environment for the formation of the reactive host-guest complex for transglycosylation. However, in the presence of a more active sialyl donor than the natural one, the mutated enzyme is able to transfer efficiently the sialyl residue to a suitable acceptor. Rational engineering of enzymes generally requires reliance on the static 3D protein structure. These altered enzymes may be devoid of product hydrolysis activity.

A second technique called directed evolution strategy comprises random mutagenesis of the selected natural sialidase enzyme thus creating a library of enzyme variants each of which are altered in a single or multiple positions. They may be inserted into suitable microorganisms such as *E. coli* or *S. cerevisiae* for producing recombinant variants with slightly altered properties. Clones expressing improved enzymes are then identified with a fast and reliable screening method, selected and brought into a next round of the mutation process. The recurring cycles of mutation, recombination and selection are continued as far as mutant(s) with the desired activity and/or specificity is/are evolved.

With regard to transsialidases, the first transsialidase enzyme described was found in *Trypanosoma cruzi*, a protozoa which causes Chagas disease. Since that time transsialidases have been detected in several other trypanosome types such as *Trypanosoma brucei gambiense*, *Trypanosoma brucei rhodesiense*, *Trypanosoma brucei brucei* and *Trypanosoma congolense*. Moreover, the existence of transsialidases has been shown in *Endotrypanum* types, in *Corynebacterium diphtheriae* and even in the human plasma.

Transsialidases differ from sialidases in that, in addition to the hydrolytic activity towards sialic acids of the sialidases, the former have more considerable sialic acid transfer activity. Transsialidases can transfer sialic acids, preferably α -2,3-bonded sialic acids, from a donor molecule to an acceptor derivative, which is preferably a terminal galactose moiety with β -interglycosidic linkage. As a result of this transfer, an α -glycosidic bond is formed between the sialic acid and the acceptor. However, if there is no suitable acceptor, the transsialidase hydrolyses the sialic acid.

It is possible to produce directed transsialidase enzyme mutants wherein the hydrolase activity is effaced in favour of the transsialidase action, e.g. by altering the amino acid sequence. After creating a library of altered genes by mutagenesis and/or recombination, they may be inserted into suitable microorganisms such as *E. coli* or *S. cerevisiae* for producing recombinant variants with slightly altered properties. Clones expressing improved enzymes are then identified, isolated and can be used for the desired purpose. For example, based on sequence and structure comparisons, sialidase from *Trypanosoma rangeli* may be mutated at six positions, wherein the resulting mutant is able to display a significant level of trans-sialidase activity (Paris et al. *J. Mol. Biol.* 345, 923 (2005)).

Preferably, the enzyme having transsialidase activity may be selected from sialidases or transsialidases derived from *Bifidobacterium longum* subsp. *infantis* ATCC 15697, *Bifidobacterium bifidum* JCM1254, *Bifidobacterium bifidum* S17, *Bifidobacterium bifidum* PRL2010, *Bifidobacterium bifidum* NCIMB 41171, *Trypanosoma cruzi*, etc.

More preferably the enzyme having transsialidase activity may be selected from sialidases or transsialidases as defined according to the following deposit numbers: gi|213524659 (10) (*Bifidobacterium longum* subsp. *infantis* ATCC 15697, SEQ ID NO: 1), gi|213523006 (*Bifidobacterium longum* subsp. *infantis* ATCC 15697, SEQ ID NO: 2), gi|309252191 (15) (*Bifidobacterium bifidum* S17, SEQ ID NO: 3), gi|309252190 (*Bifidobacterium bifidum* S17, SEQ ID NO: 4), gi|310867437 (*Bifidobacterium bifidum* PRL2010, SEQ ID NO: 5), gi|310867438 (*Bifidobacterium bifidum* PRL2010, SEQ ID NO: 6), gi|224283484 (20) (*Bifidobacterium bifidum* NCIMB 41171, SEQ ID NO: 7), gi|224283485 (*Bifidobacterium bifidum* NCIMB 41171, SEQ ID NO: 8), gi|334283443 gi|47252690 (*Bifidobacterium bifidum* JCM1254, SEQ ID NO: 9), gi|47252690 (*T. cruzi*, SEQ ID NO: 10), gi|432485 (25) (*T. cruzi*, SEQ ID NO: 11). Particularly preferred sialidases/transsialidases with transsialidase activity are listed in the following Table 2:

TABLE 2

Preferred sialidases/transsialidases		
GI number in GenBank Database	Organism	SEQ ID NO:
gi 213524659	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> ATCC 15697	1
gi 213523006	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> ATCC 15697	2
gi 309252191	<i>Bifidobacterium bifidum</i> S17	3
gi 309252190	<i>Bifidobacterium bifidum</i> S17	4
gi 310867437	<i>Bifidobacterium bifidum</i> PRL2010	5
gi 310867438	<i>Bifidobacterium bifidum</i> PRL2010	6
gi 224283484	<i>Bifidobacterium bifidum</i> NCIMB 41171	7
gi 224283485	<i>Bifidobacterium bifidum</i> NCIMB 41171	8
gi 334283443	<i>Bifidobacterium bifidum</i> JCM1254	9
gi 47252690	<i>Trypanosoma cruzi</i>	10
gi 432485	<i>Trypanosoma cruzi</i>	11

It is envisaged that sialidase/transsialidase enzyme mutants retaining transsialidase activity and having a sequence similarity/homology to the sequence of the above mentioned enzyme sequences having transsialidase activity of at least 70%, more preferably at least 80%, even more preferably at least 85%, even more preferably at least 90% and most preferably at least 95% or even 97%, 98% or 99% as compared to the entire wild type sequence on amino acid level.

Preferably, the sequence similarity is at least 90%, more preferably 95%, 97%, 98% or most preferably 99%. Preferably, said trans-sialidase activity is at least 75% of that of the native form of the enzyme, more preferably at least 90% and still more preferably at least 100%.

Sialidases and transsialidases possess a broader donor and acceptor specificity than the sialyl transferases used in prior art processes, and so can be used in a particularly wide variety of reactions. Sialidases/transsialidases are therefore more advantageous for industrial utilisation than are sialyltransferases previously used.

65 Donors for Sialidases/Transsialidases

It is known that, upon infection of an organism with *T. cruzi*, the transsialidase in *T. cruzi* scavenges sialic acids from

11

sialoglycoconjugates of the host's organism and efficiently sialylates its own surface mucin in order to mask its own epitope.

After numerous intensive investigations it has been found that a huge number of natural and synthetic sialic acid containing derivatives can act as sialyl donors in transsialidation reactions. Thus sialyl donor compounds of general formula SA-OR² defined above can provide a substrate to be transferred by transsialidases to the acceptor. Transsialidases do not transfer pure sialic acid or CMP-sialic acid (which is, in fact, a β -sialide) to the acceptor, the presence of a sialic acid with an α -anomeric aglycon or α -anomeric substituent is a requisite for the transsialidase reactions. Typical natural sialyl donors can be selected from, but are not limited to, 3'-O-sialyl-lactose, fetuin, gangliosides, O- or N-linked glycopeptides, all of which contain a sialic acid α -2,3-linked to a terminal β -galactoside residue, or polysialic acid with α -2,8-linkage. Among synthetic sialosides 2-O-(4-methylumbelliferyl)- or 2-O-(optionally substituted phenyl)- α -D-sialosides, more commonly 2-O-(p-nitrophenyl)- α -D-sialoside, are of preference.

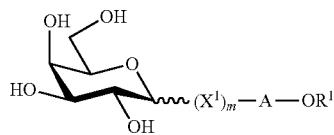
Acceptors for Sialidases/Transsialidases

Transsialidase acceptors used in transsialidation reaction disclosed above are characterized by general formula 2 and salts thereof which are R¹-galactopyranosides (when n is 0) or oligosaccharide R¹-glycosides whose terminal sugar moiety on the non-reducing end is galactopyranose (when n equals 1). The terminal galactopyranosyl unit is bound preferably with a β -glycosidic linkage.

The anomeric hydroxyl group of compounds of general formula 2 are protected with R¹ groups that can be removed by hydrogenolysis. As set out previously, such groups include optionally substituted benzyl and naphthylmethyl groups. Benzyl or 2-naphthylmethyl groups optionally substituted with phenyl, alkyl or halogen are preferred R¹-groups, and among them unsubstituted benzyl, unsubstituted 2-naphthylmethyl, 4-chlorobenzyl, 3-phenylbenzyl or 4-methylbenzyl groups are of particular preference.

When n is 1, the structural element X, as carbohydrate linker, means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure. The monosaccharide building units of the carbohydrate linker can be any naturally occurring 5-, 6- or 9-carbon containing sugar derivatives, with the most frequently occurring units being glucose, N-acetyl glucosamine, galactose, fucose and sialic acid.

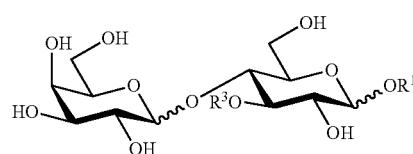
In a preferred method n is 1 and linker X corresponds to formula —(X¹)_m-A-forming acceptors of general formula 2A or salts thereof



wherein A is a D-glucopyranosyl unit optionally substituted with fucosyl, X¹ represents a carbohydrate linker, and integer m is 0 or 1. Group —OR¹ is linked to the anomeric carbon (C₁) atom of the D-glucopyranosyl ring, preferably in β orientation.

When m is 0, the terminal D-galactopyranosyl unit is directly coupled to group A through an interglycosidic linkage. Preferably, the interglycosidic linkage is a 1-4 linkage thus forming compounds of general formula 2B

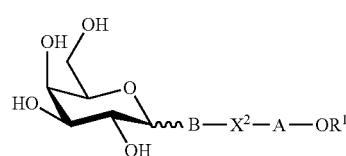
12



2B

wherein R³ is fucosyl or H. More preferably the interglycosidic linkage between the galactose and the glucose portion is β thus giving rise to a lactose derivative. In an even more preferable embodiment aglycon —OR¹ is also in β orientation.

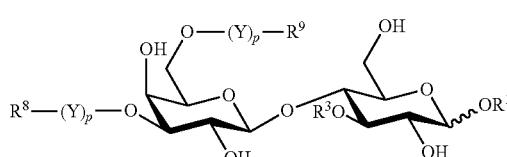
When m is 1, the structural element X¹, as carbohydrate linker, means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure. The monosaccharide building units of the carbohydrate linker can be any naturally occurring 5-, 6- or 9-carbon containing sugar derivatives, with the most frequently occurring units being glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Preferably, linker X¹ is represented by the formula —B—X²— thus forming compounds of general formula 2C or salts thereof



2C

wherein group B is an N-acetyl-glucosaminopyranosyl unit optionally substituted with fucosyl and/or sialyl, and linker X² means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure having the monosaccharide building units selected from glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Group —OR¹ is linked to the anomeric carbon (C₁) atom of the D-glucopyranosyl ring, preferably in β orientation. In preference, the terminal galactosyl group is attached to group B through 1-3 or 1-4 interglycosidic linkage forming thus a lacto-N-biosyl or N-acetyl-lactosaminyl terminal disaccharide moieties, respectively. In a further favoured method, a fucosyl substituent may be coupled to the 3-OH or 4-OH group of unit B and/or to the 3-OH group of unit A, and/or sialyl may be connected to 6-OH of unit B.

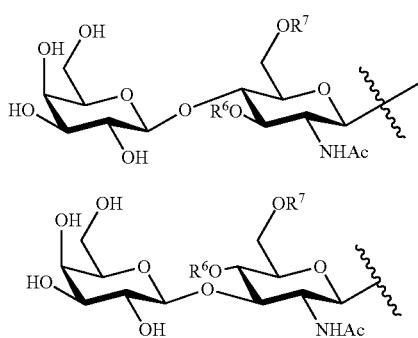
In a more preferred method group X² is galactose optionally substituted with sialyl or oligosaccharide representing a linear or a branched structure having the saccharide building units selected from N-acetyl-lactosamine, lacto-N-biose, fucose and sialic acid, forming thus human milk oligosaccharide derivatives represented by general formula 2D or salts thereof



2D

13

wherein R¹ is a group removable by hydrogenolysis, R³ is H or fucosyl unit, Y is independently an N-acetyl-lactosaminyl group optionally substituted with a sialyl and/or fucosyl residue, integer p is independently 0, 1 or 2, R⁸ is selected from the groups characterized by general formulae 5 and 6,



wherein R⁶ is H or fucosyl residue, R⁷H or α-sialyl moiety, and R⁹ is selected from H, α-sialyl moiety, a group of general formula 5 and a group of general formula 6.

According to a further preferred method, the compound of general formula 2D and salts thereof as defined above is characterized by its linkages and attached moieties, wherein an N-acetyl-lactosaminyl group in group Y, when attached to another N-acetyl-lactosaminyl group (p=2), is coupled with 1-3 interglycosidic linkage, the group of general formula 5, when attached to Y (p=1, 2), is coupled with 1-3 interglycosidic linkage, the group of general formula 6, when attached to Y (p=1, 2), is coupled with 1-3 interglycosidic linkage, the fucosyl residue if attached to a N-acetyl-lactosaminyl group present in Y is linked to the N-acetyl-glucosamine of the N-acetyl-lactosaminyl group with 1-3 interglycosidic linkage, the α-sialyl residue if attached to a N-acetyl-lactosaminyl group present in Y is linked to the galactose of the N-acetyl-lactosaminyl group with 2-6 interglycosidic linkage.

In a further aspect, the compound of general formula 2B, 2C or 2D and salts thereof as defined above represents the R¹-glycosides of lactose, lacto-N-neotetraose, para-lacto-N-hexaose, para-lacto-N-neohexaose, lacto-N-neohexaose, para-lacto-N-octaose and lacto-N-neooctaose, lacto-N-tetraose, lacto-N-hexaose, lacto-N-octaose, iso-lacto-N-octaose, lacto-N-decaose and lacto-N-neodecaose optionally substituted with one or more sialyl and/or fucosyl residue and having unsubstituted terminal galactosyl residue. Preferably, the sialyl substituent is N-acetyl neuraminy group.

Particularly preferably, the compound of general formula 2B, 2C or 2D and salts thereof as defined above is selected from the group of R¹-glycosides of Galβ1-4Glc (lactose), Galβ1-4(Fucα1-3)Glc (3-O-fucosyllactose), Galβ1-3GlcNAcβ1-3Galβ1-4Glc (LNT), Galβ1-4GlcNAcβ1-3Galβ1-4Glc (LNNT), Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc (LNFP II), Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc (LNFP III), Galβ1-3GlcNAcβ1-3Galβ1-4(Fucα1-4)Glc (LNFP V), Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc (LNDFH II), Galβ1-3(Neu5Aca2-6)GlcNAcβ1-3Galβ1-4Glc (LSTb), Galβ1-3(Neu5Aca2-6)(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc, Galβ1-3(Neu5Aca2-6)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc, Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc, Galβ1-4(Fucα1-3)GlcNAcβ1-

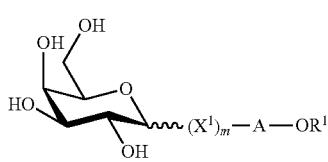
14

3Galβ1-4(Fucα1-3)Glc (LNDFH III), or salts thereof. The R¹-glycosides may be alpha or beta-anomers. Preferably, said R¹-glycosides are the beta-anomers.

A typical synthesis of compounds of general formula 2 comprises the treatment of galactose, or oligosaccharides having a galactopyranosyl unit at the nonreducing terminal, with acetic anhydride and sodium acetate at 50-125° C., followed by Lewis acid catalyzed glycosylation using R¹-OH, preferably benzyl/substituted benzyl alcohols, in organic solvent such as DCM, toluene, THF, etc. Subsequently, compounds of general formula 2 are obtained via a final Zemplén deprotection of the glycosylated products.

According to another typical anomeric O-protection procedure, fully or partially protected galactose or oligosaccharides having a galactopyranosyl unit at the nonreducing end with a free anomeric OH in a dipolar aprotic solvent such as DMF, DMSO, N-methylpyrrolidone, hexamethylphosphoramide (HMPA), N,N'-dimethylhexahydronimidine-2-one (DMPU), THF, dioxane, acetonitrile, etc., or mixture thereof, is O-alkylated in the presence of a strong base and R¹-X wherein X is a leaving group selected from halogen, alkylsulfonyloxy like mesyl, triflyl, etc. and arylsulfonyl like benzenesulfonyl, tosyl, etc. Preferred alkylating agents are benzyl or 1- or 2-naphthylmethyl halogenides optionally substituted with one or more groups selected from phenyl, alkyl or halogen. The strong base is able to deprotonate the anomeric OH chemoselectively due to its more acidic character when an equivalent amount or a slight excess (1 to 1.5 equiv.) of base is used. The strong base suitable for activating the anomeric OH is typically taken from the group of alkali metal or alkaline earth metal hydrides or alkoxides such as NaH, KH, CaH₂, NaOMe, NaOBu, KOBu, inorganic hydroxides, potassium carbonate, etc. The alkylation agent is added in an equivalent amount or a slight excess (1 to 1.5 equiv.). The reaction is carried out between -10 and 80° C., preferably at a low temperature during whole course of the reaction or at a low temperature during the addition of the reagents/reactants and an elevated temperature in the later stages of the course of the reaction. Benzyl/substituted benzyl glycosides of general formula 2 can be obtained after usual work-up.

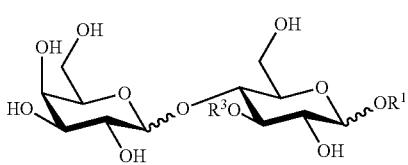
In a further aspect, the present invention relates to providing compounds of general formula 2A' and salts thereof



wherein A is a D-glucopyranosyl unit optionally substituted with fucosyl, X¹ represents a carbohydrate linker, integer m is 0 or 1, provided that a) when m is 0, then group A is substituted with fucosyl, and b) the compound differs to 1-O-β-benzyl-LNT, 1-O-β-(4-hydroxymethylbenzyl)-LNNT and 1-O-β-benzyl-LNNT. Group —OR¹ is linked to the anomeric carbon (C₁) atom of the D-glucopyranosyl ring, preferably in β orientation.

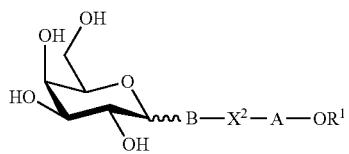
When m is 0, the terminal D-galactopyranosyl unit is directly coupled to group A through an interglycosidic linkage. Preferably, the interglycosidic linkage is a 1-4 linkage thus forming compounds of general formula 2B' and salts thereof

15



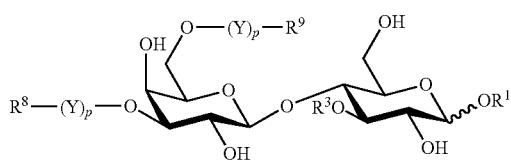
wherein R^3 is fucosyl. More preferably the interglycosidic linkage between the galactose and the glucose portion is β thus giving rise to a lactose derivative. In an even more preferable embodiment aglycon —OR¹ is also in β orientation.

When m is 1, the structural element X¹, as carbohydrate linker, means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure. The monosaccharide building units of the carbohydrate linker can be any naturally occurring 5-, 6- or 9-carbon containing sugar derivatives, with the most frequently occurring units being glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Preferably, linker X¹ is represented by the formula —B—X²— thus forming compounds of general formula 2C' and salts thereof



wherein group B is an N-acetyl-glucosaminopyranosyl unit optionally substituted with fucosyl and/or sialyl, and linker X² means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure having the monosaccharide building units selected from glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Group —OR¹ is linked to the anomeric carbon (C₁) atom of the D-glucopyranosyl ring, preferably in β orientation. In preference, the terminal galactosyl group is attached to group B through 1-3 or 1-4 interglycosidic linkage forming thus a lacto-N-biosyl or N-acetyl-lactosaminyl terminal disaccharide moieties, respectively. In a further favoured method, a fucosyl substituent may be coupled to the 3-OH or 4-OH group of unit B and/or to the 3-OH group of unit A, and/or sialyl may be connected to 6-OH of unit B.

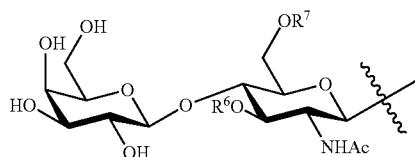
In a more preferred method group X² is galactose optionally substituted with sialyl or oligosaccharide residue representing a linear or a branched structure having the saccharide building units selected from N-acetyl-lactosamine, lacto-N-biose, fucose and sialic acid, forming thus human milk oligosaccharide derivatives represented by general formula 2D' or salts thereof.



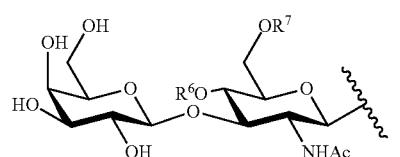
wherein R¹ is a group removable by hydrogenolysis, R³ is H or fucosyl unit, Y is independently an N-acetyl-lactosami-

2B'

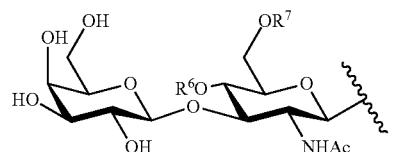
5



nyl group optionally substituted with a sialyl and/or fucosyl residue, integer p is independently 0, 1 or 2, R⁸ is selected from the groups characterized by general formulae 5 and 6,



5



6

wherein R⁶ is H or fucosyl residue, R⁷H or α -sialyl moiety, and R⁹ is selected from H, α -sialyl moiety, a group of general formula 5 and a group of general formula 6.

According to a further preferred embodiment, the compound of general formula 2D' and salts thereof as defined above is characterized by its linkages and attached moieties, wherein

an N-acetyl-lactosaminyl group in group Y, when attached to another N-acetyl-lactosaminyl group, is coupled with 1-3 interglycosidic linkage,

the group of general formula 5, when attached to Y, is coupled with 1-3 interglycosidic linkage,

the group of general formula 6, when attached to Y, is coupled with 1-3 interglycosidic linkage,

the fucosyl residue if attached to a N-acetyl-lactosaminyl group present in Y is linked to the N-acetyl-glucosamine of the N-acetyl-lactosaminyl group with 1-3 interglycosidic linkage,

the α -sialyl residue if attached to a N-acetyl-lactosaminyl group in Y is linked to the galactose of the N-acetyl-lactosaminyl group with 2-6 interglycosidic linkage.

In a further aspect, the compound of general formula 2C' or 2D' and salts thereof as defined above represents the R¹-glycosides lacto-N-neotetraose, para-lacto-N-hexaose, para-lacto-N-neohexaose, lacto-N-neohexaose, para-lacto-N-octaose and lacto-N-neooctaose, lacto-N-tetraose, lacto-N-hexaose, lacto-N-octaose, iso-lacto-N-octaose, lacto-N-decaose and lacto-N-neodecaose optionally substituted with one or more sialyl and/or fucosyl residue and having unsubstituted terminal galactosyl residue. Preferably, the sialyl substituent is N-acetyl neuraminy group.

Particularly preferably, the compound of general formula 2A' and salts thereof as defined above is selected from the group of R¹-glycosides of Gal β 1-4(Fuc α 1-3)Glc (3-O-fucosyl lactose), Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc (LNT), Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc (LNNT), Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc (LNFP II), Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc (LNFP III), Gal β 1-3GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc (LNFP V), Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc (LNDFH II), Gal β 1-3(Neu5Acc α 2-6)GlcNAc β 1-3Gal β 1-4Glc (LSTb), Gal β 1-3(Neu5Acc α 2-6)(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc, Gal β 1-3(Neu5Acc α 2-6)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Gal β 1-4GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc (LNDFH III), or salts thereof. The R¹-glycosides may be alpha or beta-anomers. Preferably, said R¹-glycosides are the beta-anomers.

2D'

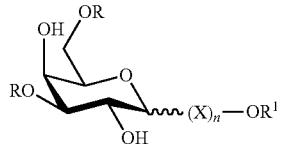
60

wherein R¹ is a group removable by hydrogenolysis, R³ is H or fucosyl unit, Y is independently an N-acetyl-lactosami-

17

Products of Transsialidation Reaction

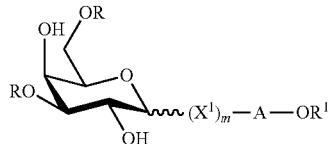
As set forth above, the transsialidation reaction claimed in the present application produces compounds of general formula 1 and salts thereof



wherein one of the R groups is an α -sialyl moiety and the other is H, X represents a carbohydrate linker, R^1 is a protecting group that is removable by hydrogenolysis and the integer n is 0 or 1, from compounds of general formula 2 and salts thereof. R^1 group includes optionally substituted benzyl and naphthylmethyl groups, among which benzyl or 2-naphthylmethyl groups optionally substituted with phenyl, alkyl or halogen are preferred R^1 -groups, and among them unsubstituted benzyl, unsubstituted 2-naphthylmethyl, 4-chlorobenzyl, 3-phenylbenzyl or 4-methylbenzyl groups are of particular preference.

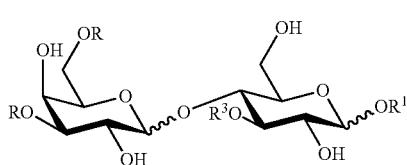
When n is 1, the structural element X, as carbohydrate linker, means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure. The monosaccharide building units of the carbohydrate linker can be any naturally occurring 5-, 6- or 9-carbon containing sugar derivatives, with the most frequently occurring units being glucose, N-acetyl glucosamine, galactose, fucose and sialic acid. Preferably, linker X¹ is represented by the formula —B—X²— thus forming compounds of general formula 1C and salts thereof

In a preferred method n is 1 and linker X corresponds to formula —(X¹)_m-A-forming sialylated products of general formula 1A and salts thereof



wherein A is a D-glucopyranosyl unit optionally substituted with fucosyl, X¹ represents a carbohydrate linker, and integer m is 0 or 1. Group —OR¹ is linked to the anomeric carbon (C₁) atom of the D-glucopyranosyl ring, preferably in β orientation.

When m is 0, the D-galactopyranosyl unit is directly coupled to group A through an interglycosidic linkage. Preferably, the interglycosidic linkage is a 1-4 linkage thus forming compounds of general formula 1B and salts thereof.

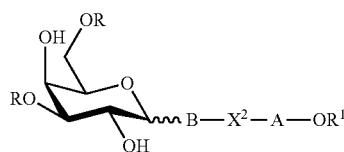


wherein R³ is fucosyl or H. More preferably the interglycosidic linkage between the galactose and the glucose portion

18

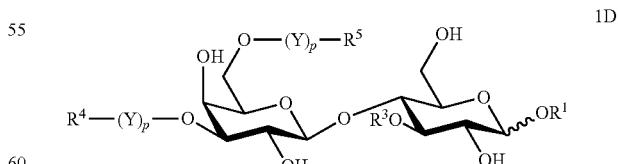
is β thus giving rise to a 3'-O-sialyl-lactose derivative. In an even more preferable embodiment aglycon —OR¹ is also in β orientation.

When m is 1, the structural element X¹, as carbohydrate linker, means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure. The monosaccharide building units of the carbohydrate linker can be any naturally occurring 5-, 6- or 9-carbon containing sugar derivatives, with the most frequently occurring units being glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Preferably, linker X¹ is represented by the formula —B—X²— thus forming compounds of general formula 1C and salts thereof



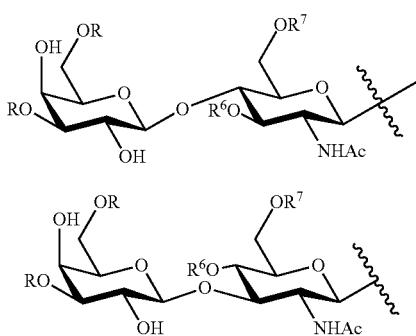
wherein group B is an N-acetyl-glucosaminopyranosyl unit optionally substituted with fucosyl and/or sialyl, and linker X² means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure having the monosaccharide building units selected from glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Group —OR¹ is linked to the anomeric carbon (C₁) atom of the D-glucopyranosyl ring, preferably in β orientation. In preference, the sialyl-galactosyl group is attached to group B through 1-3 or 1-4 interglycosidic linkage forming thus a sialyl-lacto-N-biosyl or sialyl-N-acetyl-lactosaminyl terminal trisaccharide moieties, respectively. In a further favoured method, a fucosyl substituent may be coupled to the 3-OH or 4-OH group of unit B and/or to the 3-OH group of unit A, and/or sialyl may be connected to 6-OH of unit B.

In a more preferred method to synthesize sialyl oligosaccharide derivatives, group X² in compounds of general formula 1C and salts thereof is galactose optionally substituted with sialyl or oligosaccharide representing a linear or a branched structure having the saccharide building units selected from N-acetyl-lactosamine, lacto-N-biose, fucose and sialic acid, forming thus human milk oligosaccharide derivatives represented by general formula 1D and salts thereof



wherein R¹ is a group removable by hydrogenolysis, R³ is H or fucosyl unit, Y is independently N-acetyl-lactosaminyl group optionally substituted with a sialyl and/or fucosyl residue, integer p is independently 0, 1 or 2, R⁴ is selected from the groups characterized by general formulae 3 and 4,

19



wherein R^6 is H or fucosyl residue, R^7 H or α -sialyl moiety, one of the R groups is an α -sialyl moiety and the other is H, and R^5 is selected from H, α -sialyl moiety, group of general formula 3 and group of general formula 4.

According to a further preferred method, the compound of general formula 1D and salts thereof as defined above is characterized by its linkages and attached moieties, wherein an N-acetyl-lactosaminyl group in group Y ($p=2$), when attached to another N-acetyl-lactosaminyl group, is coupled with 1-3 interglycosidic linkage, the group of general formula 3, when attached to Y ($p=1$, 2), is coupled with 1-3 interglycosidic linkage, the group of general formula 4, when attached to Y ($p=1$, 2), is coupled with 1-3 interglycosidic linkage, the fucosyl residue if attached to a N-acetyl-lactosaminyl group present in Y is linked to the N-acetyl-glucosamine of the N-acetyl-lactosaminyl group with 1-3 interglycosidic linkage, the α -sialyl residue if attached to a N-acetyl-lactosaminyl group in Y is linked to the galactose of the N-acetyl-lactosaminyl group with 2-6 interglycosidic linkage.

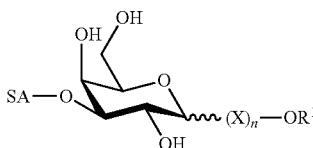
In a further aspect, the compound of general formula 1B, 1C or 1D and salts thereof as defined above represents the R^4 -glycosides of lactose, lacto-N-neotetraose, para-lacto-N-hexaose, para-lacto-N-neohexaose, lacto-N-neohexaose, para-lacto-N-octaose and lacto-N-neooctaose, lacto-N-tetraose, lacto-N-hexaose, lacto-N-octaose, iso-lacto-N-octaose, lacto-N-decaose and lacto-N-neodecaose optionally substituted with one or more sialyl and/or fucosyl residue and having sialyl substituent in 3-OH or 6-OH of a terminal galactosyl residue. Preferably, the sialyl substituent(s) is/are N-acetyl-neuraminyl group(s).

Particularly preferably, the compound of general formula 1B, 1C or 1D and salts thereof as defined above is selected from the group of R^4 -glycosides of Neu5Aco2-3Gal β 1-4Glc (3'-O-(N-acetyl-neuraminosyl)-lactose), Neu5Aco2-6Gal β 1-4Glc (6'-O-(N-acetyl-neuraminosyl)-lactose), Neu5Aco2-3Gal β 1-4(Fuc α 1-3)Glc (3-O-fucosyl-3'-O-(N-acetyl-neuraminosyl)-lactose), Neu5Aco2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc (LST a), Neu5Aco2-6Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc, Neu5Aco2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc, Neu5Aco2-6Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc (LST c), Neu5Aco2-3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc (FLST a), Neu5Aco2-6Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc, Neu5Aco2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc, Neu5Aco2-Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc, Neu5Aco2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Aco2-6Gal β 1-3GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Aco2-6Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc,

20

(Fuc α 1-3)Glc, Neu5Aco2-3Gal β 1-3 (Neu5Aco2-6) GlcNAc β 1-3Gal β 1-4Glc (DSLNT), Neu5Aco2-6Gal β 1-3 (Neu5Aco2-6)GlcNAc β 1-3Gal β 1-4Glc, Neu5Aco2-3Gal β 1-3(Neu5Aco2-6)(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc (FD-DSLNT I), Neu5Aco2-6Gal β 1-3(Neu5Aco2-6)(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc, Neu5Aco2-3Gal β 1-3(Neu5Aco2-6)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc (FD-DSLNT II), Neu5Aco2-6Gal β 1-3(Neu5Aco2-6)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Aco2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Aco2-6Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Aco2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc, Neu5Aco2-6Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc or salts thereof. The R^1 -glycosides may be alpha- or beta-anomers. Preferably, said R^1 -glycosides are the beta-anomers.

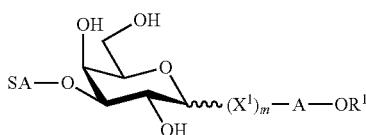
In case of selective α -2-3 sialidation, the transsialidation reaction claimed in the present application produces compounds of general formula 1-3 and salts thereof



30 wherein SA is an α -sialyl moiety, X represents a carbohydrate linker, R^1 is a protecting group that is removable by hydrogenolysis and the integer n is 0 or 1, from compounds of general formula 2 and salts thereof. R^1 group includes optionally substituted benzyl and naphthylmethyl groups, among which benzyl or 2-naphthylmethyl groups optionally substituted with phenyl, alkyl or halogen are preferred R^1 -groups, and among them unsubstituted benzyl, unsubstituted 2-naphthylmethyl, 4-chlorobenzyl, 3-phenylbenzyl or 4-methylbenzyl groups are of particular preference.

40 When n is 1, the structural element X, as carbohydrate linker, means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure. The monosaccharide building units of the carbohydrate linker can be any naturally occurring 5-, 6- or 9-carbon containing sugar derivatives, with the most frequently occurring units being glucose, N-acetyl glucosamine, galactose, fucose and sialic acid.

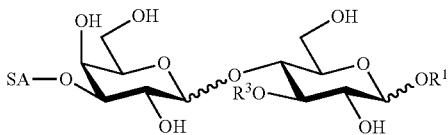
45 In a preferred method n is 1 and linker X corresponds to formula $-(X^1)_m-A-$ forming sialylated products of general formula 1-3A and salts thereof



50 1-3A wherein A is a D-glucopyranosyl unit optionally substituted with fucosyl, X^1 represents a carbohydrate linker, and integer m is 0 or 1. Group $-\text{OR}^1$ is linked to the anomeric carbon (C_1) atom of the D-glucopyranosyl ring, preferably in β orientation.

55 When m is 0, the D-galactopyranosyl unit is directly coupled to group A through an interglycosidic linkage. Preferably, the interglycosidic linkage is a 1-4 linkage thus forming compounds of general formula 1-3B and salts thereof

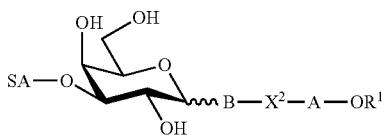
21



1-3B

wherein R³ is fucosyl or H. More preferably the interglycosidic linkage between the galactose and the glucose portion is β thus giving rise to a 3'-O-sialyl-lactose derivative. In an even more preferable embodiment aglycon —OR¹ is also in β orientation.

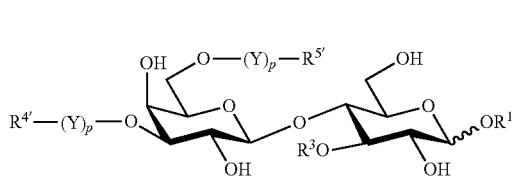
When m is 1, the structural element X¹, as carbohydrate linker, means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure. The monosaccharide building units of the carbohydrate linker can be any naturally occurring 5-, 6- or 9-carbon containing sugar derivatives, with the most frequently occurring units being glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Preferably, linker X¹ is represented by the formula —B—X²— thus forming compounds of general formula 1-3C and salts thereof



1-3C

wherein group B is an N-acetyl-glucosaminopyranosyl unit optionally substituted with fucosyl and/or sialyl, and linker X² means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure having the monosaccharide building units selected from glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Group —OR¹ is linked to the anomeric carbon (C₁) atom of the D-glucopyranosyl ring, preferably in β orientation. In preference, the sialyl-galactosyl group is attached to group B through 1-3 or 1-4 interglycosidic linkage forming thus a sialyl-lacto-N-biosyl or sialyl-N-acetyl-lactosaminyl terminal trisaccharide moieties, respectively. In a further favoured method, a fucosyl substituent may be coupled to the 3-OH or 4-OH group of unit B and/or to the 3-OH group of unit A, and/or sialyl may be connected to 6-OH of unit B.

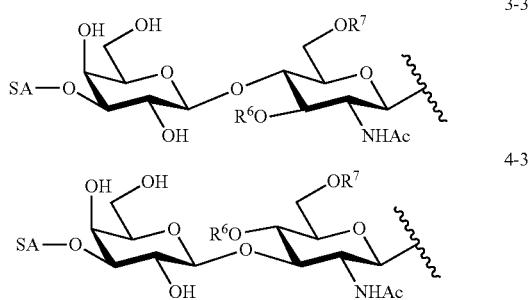
In a more preferred method to synthesize sialyl oligosaccharide derivatives, group X² in compounds of general formula 1-3C and salts thereof is galactose optionally substituted with sialyl or oligosaccharide representing a linear or a branched structure having the saccharide building units selected from N-acetyl-lactosamine, lacto-N-biose, fucose and sialic acid, forming thus human milk oligosaccharide derivatives represented by general formula 1-3D and salts thereof



1-3D

22

wherein R¹ is a group removable by hydrogenolysis, R³ is H or fucosyl unit, Y is independently N-acetyl-lactosaminyl group optionally substituted with a sialyl and/or fucosyl residue, integer p is independently 0, 1 or 2, R⁴ is selected from the groups characterized by general formulae 3-3 and 4-3,



3-3

4-3

wherein R⁶ is H or fucosyl residue, R⁷H or α -sialyl moiety, SA is α -sialyl moiety and R⁵ is selected from H, α -sialyl moiety, group of general formula 3-3 and group of general formula 4-3.

According to a further preferred embodiment, the compound of general formula 1-3D and salts thereof as defined above is characterized by its linkages and attached moieties, wherein

an N-acetyl-lactosaminyl group in group Y (p=2), when attached to another N-acetyl-lactosaminyl group, is coupled with 1-3 interglycosidic linkage, the group of general formula 3-3, when attached to Y (p=1, 2), is coupled with 1-3 interglycosidic linkage, the group of general formula 4-3, when attached to Y (p=1, 2), is coupled with 1-3 interglycosidic linkage, the fucosyl residue if attached to a N-acetyl-lactosaminyl group present in Y is linked to the N-acetyl-glucosamine of the N-acetyl-lactosaminyl group with 1-3 interglycosidic linkage, the α -sialyl residue if attached to a N-acetyl-lactosaminyl present in Y is linked to the galactose of the N-acetyl-lactosaminyl group with 2-6 interglycosidic linkage.

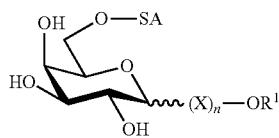
In a further aspect, the compound of general formula 1-3B, 1-3C or 1-3D and salts thereof as defined above represents the R¹-glycosides of lactose, lacto-N-neotetraose, para-lacto-N-hexaose, para-lacto-N-neohexaose, lacto-N-neohexaose, para-lacto-N-octaose and lacto-N-neooctaose, lacto-N-tetraose, lacto-N-hexaose, lacto-N-octaose, iso-lacto-N-octaose, lacto-N-decaose and lacto-N-neodecaose optionally substituted with one or more sialyl and/or fucosyl residue and having sialyl substituent in 3-OH of a terminal galactosyl residue, and salts thereof. Preferably, the sialyl substituent(s) is/are N-acetyl neuraminy group(s).

Particularly preferably, the compound of general formula 1-3B, 1-3C or 1-3D and salts thereof as defined above is selected from the group of R¹-glycosides of Neu5Ac α 2-3Gal β 1-4Glc (3'-O-(N-acetyl-neuraminosyl)-lactose), Neu5Aco2-3Gal β 1-4(Fuc α 1-3)Glc (3-O-fucosyl-3'-O-(N-acetyl-neuraminoxy)-lactose), Neu5Aco2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc (LST a), Neu5Aco2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc, Neu5Aco2-3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc (FLST a), Neu5Aco2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc, Neu5Aco2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Aco2-3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Aco2-3Gal β 1-3Gal β 1-3(Neu5Aco2-6)GlcNAc β 1-3Gal β 1-4Glc

23

(DSLNT), Neu5Ac α 2-3Gal β 1-3(Neu5Ac α 2-6)(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc (FDSSLNT I), Neu5Ac α 2-3Gal β 1-3(Neu5Ac α 2-6)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc (FDSSLNT II), Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Ac α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Ac α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc and salts thereof. The R 1 -glycosides may be alpha- or beta-anomers. Preferably, said R 1 -glycosides are the beta-anomers.

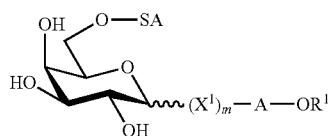
In case of selective α -2-6 sialidation, the transsialidation reaction claimed in the present application produces compounds of general formula 1-6 and salts thereof



wherein SA is an α -sialyl moiety, X represents a carbohydrate linker, R 1 is a protecting group that is removable by hydrogenolysis and the integer n is 0 or 1, from compounds of general formula 2 and salts thereof. R 1 group includes optionally substituted benzyl and naphthylmethyl groups, among which benzyl or 2-naphthylmethyl groups optionally substituted with phenyl, alkyl or halogen are preferred R 1 -groups, and among them unsubstituted benzyl, unsubstituted 2-naphthylmethyl, 4-chlorobenzyl, 3-phenylbenzyl or 4-methylbenzyl groups are of particular preference.

When n is 1, the structural element X, as carbohydrate linker, means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure. The monosaccharide building units of the carbohydrate linker can be any naturally occurring 5-, 6- or 9-carbon containing sugar derivatives, with the most frequently occurring units being glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Preferably, linker X 1 is represented by the formula —B—X 2 — thus forming compounds of general formula 1-6C and salts thereof.

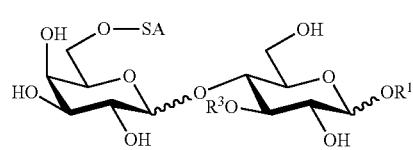
In a preferred method n is 1 and linker X corresponds to formula —(X 1) $_m$ -A-forming sialylated products of general formula 1-6A and salts thereof.



wherein A is a D-glucopyranosyl unit optionally substituted with fucosyl, X 1 represents a carbohydrate linker, and integer m is 0 or 1. Group —OR 1 is linked to the anomeric carbon (C $_1$) atom of the D-glucopyranosyl ring, preferably in β orientation.

When m is 0, the D-galactopyranosyl unit is directly coupled to group A through an interglycosidic linkage. Preferably, the interglycosidic linkage is a 1-4 linkage thus forming compounds of general formula 1-6B and salts thereof

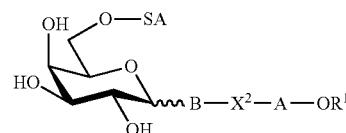
24



1-6B

wherein R 3 is fucosyl or H. More preferably the interglycosidic linkage between the galactose and the glucose portion is β thus giving rise to a 3'-O-sialyl-lactose derivative. In an even more preferable embodiment aglycon —OR 1 is also in β orientation.

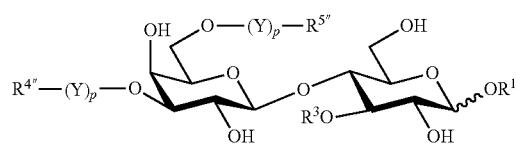
When m is 1, the structural element X 1 , as carbohydrate linker, means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure. The monosaccharide building units of the carbohydrate linker can be any naturally occurring 5-, 6- or 9-carbon containing sugar derivatives, with the most frequently occurring units being glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Preferably, linker X 1 is represented by the formula —B—X 2 — thus forming compounds of general formula 1-6C and salts thereof.



1-6C

wherein group B is an N-acetyl-glucosaminopyranosyl unit optionally substituted with fucosyl and/or sialyl, and linker X 2 means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure having the monosaccharide building units selected from glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Group —OR 1 is linked to the anomeric carbon (C $_1$) atom of the D-glucopyranosyl ring, preferably in β orientation. In preference, the sialyl-galactosyl group is attached to group B through 1-3 or 1-4 interglycosidic linkage forming thus a sialyl-lacto-N-biosyl or sialyl-N-acetyl-lactosaminyl terminal trisaccharide moieties, respectively. In a further favoured method, a fucosyl substituent may be coupled to the 3-OH or 4-OH group of unit B and/or to the 3-OH group of unit A, and/or sialyl may be connected to 6-OH of unit B.

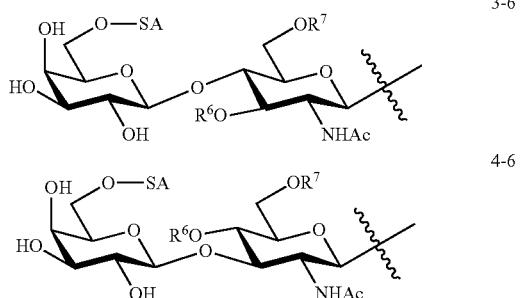
In a more preferred method to synthesize sialyl oligosaccharide derivatives, group X 2 in compounds of general formula 1-6C and salts thereof is galactose optionally substituted with sialyl or oligosaccharide representing a linear or a branched structure having the saccharide building units selected from N-acetyl-lactosamine, lacto-N-biose, fucose and sialic acid, forming thus human milk oligosaccharide derivatives represented by general formula 1-6D and salts thereof



1-6D

25

wherein R^1 is a group removable by hydrogenolysis, R^3 is H or fucosyl unit, Y is independently N-acetyl-lactosaminyl group optionally substituted with a sialyl and/or fucosyl residue, integer p is independently 0, 1 or 2, R^4 is selected from the groups characterized by general formulae 3-6 and 4-6,



wherein R^6 is H or fucosyl residue, R^7 is α -sialyl moiety, SA is α -sialyl moiety and R^5 is selected from H, α -sialyl moiety, group of general formula 3-6 and group of general formula 4-6, or salts thereof.

According to a further preferred embodiment, the compound of general formula 1-6D and salts thereof as defined above is characterized by its linkages and attached moieties, wherein

an N-acetyl-lactosaminyl group in group Y (p=2), when attached to another N-acetyl-lactosaminyl group, is coupled with 1-3 interglycosidic linkage,

the group of general formula 3-6, when attached to Y (p=1,

2), is coupled with 1-3 interglycosidic linkage, the group of general formula 4-6, when attached to Y (p=1,

2), is coupled with 1-3 interglycosidic linkage, the fucosyl residue if attached to a N-acetyl-lactosaminyl group present in Y is linked to the N-acetyl-glucosamine of the N-acetyl-lactosaminyl group with 1-3 interglycosidic linkage,

the α -sialyl residue if attached to a N-acetyl-lactosaminyl group present in Y is linked to the galactose of the N-acetyl-lactosaminyl group with 2-6 interglycosidic linkage.

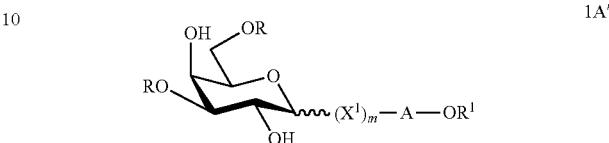
In a further aspect, the compound of general formula 1-6B, 1-6C or 1-6D and salts thereof as defined above represents the R^1 -glycosides of lactose, lacto-N-neotetraose, para-lacto-N-hexaose, para-lacto-N-neohexaose, lacto-N-neohexaose, para-lacto-N-octaose and lacto-N-neooctaose, lacto-N-tetraose, lacto-N-hexaose, lacto-N-octaose, iso-lacto-N-octaose, lacto-N-decaose and lacto-N-neodecaose optionally substituted with one or more sialyl and/or fucosyl residue and having sialyl substituent in 6-OH of a terminal galactosyl residue, and salts thereof. Preferably, the sialyl substituent(s) is/are N-acetyl neuraminyl group(s).

Particularly preferably, the compound of general formula 1-6B, 1-6C or 1-6D and salts thereof as defined above is selected from the group of R^1 -glycosides of Neu5Ac α 2-6Gal β 1-4Glc (6'-O-(N-acetyl-neuraminosyl)-lactose), Neu5Ac α 2-6Gal β 1-4(Fuca1-3)Glc, Neu5Ac α 2-6Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc (LST c), Neu5Ac α 2-6Gal β 1-3(Fuca1-4)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-6Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-6Gal β 1-3GlcNAc β 1-3Gal β 1-4(Fuca1-3)Glc, Neu5Ac α 2-6Gal β 1-3(Fuca1-4)GlcNAc β 1-3Gal β 1-4(Fuca1-3)Glc, Neu5Ac α 2-6Gal β 1-3(Neu5Ac α 2-6)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-6Gal β 1-3(Neu5Ac α 2-6)GlcNAc β 1-3(Fuca1-4)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-6Gal β 1-3(Neu5Ac α 2-6)GlcNAc β 1-3Gal β 1-4(Fuca1-3)Glc (FLST c), Neu5Ac α 2-6Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4(Fuca1-3)Glc, Neu5Ac α 2-6Gal β 1-4(Fuca1-3)GlcNAc β 1-3Gal β 1-4Glc or

26

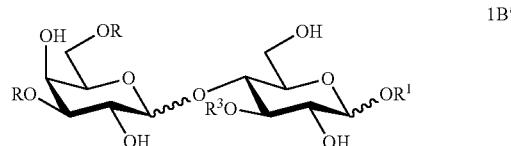
salts thereof. The R^1 -glycosides may be alpha- or beta-anomers. Preferably, said R^1 -glycosides are the beta-anomers.

It should be emphasized that some compounds represented by general formula 1 and salts thereof defined above and obtainable in the transsialidation reaction disclosed in the present application are novel. Thus the invention relates to providing novel compounds of general formula 1A' and salts thereof



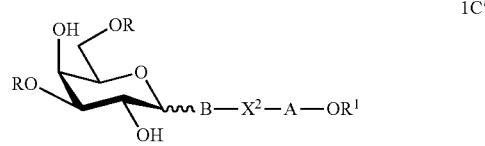
wherein one of the R groups is an α -sialyl moiety and the other is H, R^1 is a protecting group that is removable by hydrogenolysis, A is a D-glucopyranosyl unit optionally substituted with fucosyl, integer m is 0 or 1, and X^1 represents a carbohydrate linker, provided that 1-O- β -benzyl and 1-O- β -(4,5-dimethoxy-2-nitro)-benzyl glycosides of 3'-O-(N-acetyl-neuraminosyl)-lactose sodium salt, and 1-O- β -benzyl glycoside of 6'-O-(N-acetyl-neuraminosyl)-lactose sodium salt are excluded. Group $—OR^1$ is linked to the anomeric carbon (C_1) atom of the D-glucopyranosyl ring, preferably in β orientation.

When m is 0, the D-galactopyranosyl unit is directly coupled to group A through an interglycosidic linkage. Preferably, the interglycosidic linkage is a 1-4 linkage thus forming compounds of general formula 1B' and salts thereof



wherein R^3 is fucosyl or H. More preferably the interglycosidic linkage between the galactose and the glucose portion is β thus giving rise to a 3'-O-sialyl-lactose derivative. In an even more preferable embodiment aglycon $—OR^1$ is also in β orientation.

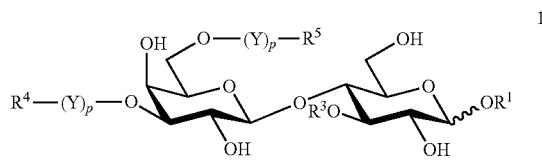
When m is 1, the structural element X^1 , as carbohydrate linker, means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure. The monosaccharide building units of the carbohydrate linker can be any naturally occurring 5-, 6- or 9-carbon containing sugar derivatives, with the most frequently occurring units being glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Preferably, linker X^1 is represented by the formula $—B—X^2—$ thus forming compounds of general formula 1C' and salts thereof.



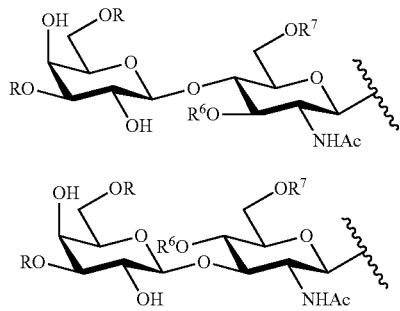
wherein group B is an N-acetyl-glucosaminopyranosyl unit optionally substituted with fucosyl and/or sialyl, and linker X^2 means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure having the monosaccharide building units selected from glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Group $—OR^1$ is linked to the anomeric carbon (C_1) atom of the D-glucopyranosyl ring, preferably in β orientation. In

preference, the sialyl-galactosyl group is attached to group B through 1-3 or 1-4 interglycosidic linkage forming thus a sialyl-lacto-N-biosyl or sialyl-N-acetyl-lactosaminyl terminal trisaccharide moieties, respectively. In a further favoured method, a fucosyl substituent may be coupled to the 3-OH or 4-OH group of unit B and/or to the 3-OH group of unit A, and/or sialyl may be connected to 6-OH of unit B.

In a more preferred embodiment, group X^2 in compounds of general formula 1C' and salts thereof is galactose optionally substituted with sialyl or oligosaccharide representing a linear or a branched structure having the saccharide building units selected from N-acetyl-lactosamine, lacto-N-biose, fucose and sialic acid, forming thus human milk oligosaccharide derivatives represented by general formula 1D' and salts thereof



wherein R^1 is a group removable by hydrogenolysis, R^3 is H or fucosyl unit, Y is independently N-acetyl-lactosaminyl group optionally substituted with a sialyl and/or fucosyl residue, integer p is independently 0, 1 or 2, R^4 is selected from the groups characterized by general formulae 3 and 4,



wherein R^6 is H or fucosyl residue, R^7 H or α -sialyl moiety, one of the R groups is an α -sialyl moiety and the other is H, and R^5 is selected from H, α -sialyl moiety, group of general formula 3 and group of general formula 4.

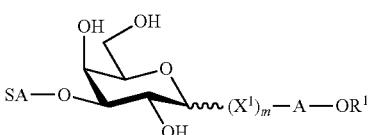
According to a further preferred embodiment, the compound of general formula 1D' and salts thereof as defined above is characterized by its linkages and attached moieties, wherein

an N-acetyl-lactosaminyl group in group Y ($p=2$), when attached to another N-acetyl-lactosaminyl group, is coupled with 1-3 interglycosidic linkage,
the group of general formula 3, when attached to Y ($p=1$, 2), is coupled with 1-3 interglycosidic linkage,
the group of general formula 4, when attached to Y ($p=1$, 2), is coupled with 1-3 interglycosidic linkage, the fucosyl residue if attached to a N-acetyl-lactosaminyl group present in Y is linked to the N-acetyl-glucosamine of the N-acetyl-lactosaminyl group with 1-3 interglycosidic linkage,
the α -sialyl residue if attached to a N-acetyl-lactosaminyl group present in Y is linked to the galactose of the N-acetyl-lactosaminyl group with 2-6 interglycosidic linkage.

In a further aspect, the compound of general formula 1B', 1C' or 1D' and salts thereof as defined above is selected from the group consisting of the R^1 -glycosides of lactose, lacto-N-neotetraose, para-lacto-N-hexaose, para-lacto-N-neohexaose, lacto-N-neohexaose, para-lacto-N-octaose and lacto-N-neooctaose, lacto-N-tetraose, lacto-N-hexaose, lacto-N-octaose, iso-lacto-N-octaose, lacto-N-decaose and lacto-N-neodecaose optionally substituted with one or more sialyl and/or fucosyl residue and having sialyl substituent in 3-OH or 6-OH of a terminal galactosyl residue, and salts thereof. Preferably, the sialyl substituent(s) is/are N-acetyl neuraminy group(s).

Particularly preferably, the compound of general formula 1A' and salts thereof as defined above is selected from the group consisting of R^1 -glycosides of Neu5Ac α 2-3Gal β 1-4Glc (3'-O-(N-acetyl-neuraminosyl)-lactose), Neu5Ac α 2-6Gal β 1-4Glc (6'-O-(N-acetyl-neuraminosyl)-lactose), Neu5Ac α 2-3Gal β 1-4(Fuc α 1-3)Glc (3-O-fucosyl-3'-O-(N-acetyl-neuraminosyl)-lactose), Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc (LST a), Neu5Ac α 2-3GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc (LST c), Neu5Ac α 2-3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc (FLST a), Neu5Ac α 2-6Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4(Fuc α 1-4)Glc, Neu5Ac α 2-6Gal β 1-3GlcNAc β 1-3Gal β 1-4(Fuc α 1-4)Glc, Neu5Ac α 2-3Gal β 1-3Gal β 1-4(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-6Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-6Gal β 1-3(Fuc α 1-3)Glc, Neu5Ac α 2-3Gal β 1-3(GlcNAc β 1-3Gal β 1-4Glc (DSLNT), Neu5Ac α 2-6Gal β 1-3(Neu5Ac α 2-6)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4(Fuc α 1-4)Glc, Neu5Ac α 2-3Gal β 1-3Gal β 1-4(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Ac α 2-6Gal β 1-3(Fuc α 1-3)Glc, Neu5Ac α 2-3Gal β 1-3(GlcNAc β 1-3Gal β 1-4Glc (FD-SLNT I), Neu5Ac α 2-6Gal β 1-3(Neu5Ac α 2-6)Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc (FLST c), Neu5Ac α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Ac α 2-6Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Ac α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Ac α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-6Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc or salts thereof. The R^1 -glycosides may be alpha- or beta-anomers. Preferably, said R^1 -glycosides are the beta-anomers.

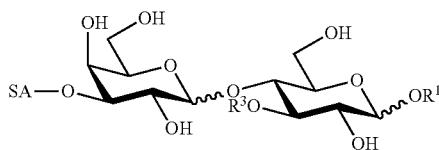
In one embodiment within the group of compounds of general formula 1A' and salts thereof the present invention relates to α -2-3-sialylated compounds of general formula 1'-3A and salts thereof



wherein A is a D-glucopyranosyl unit optionally substituted with fucosyl, X^1 represents a carbohydrate linker, and integer m is 0 or 1. Group —OR¹ is linked to the anomeric carbon (C₁) atom of the D-glucopyranosyl ring, preferably in β orientation.

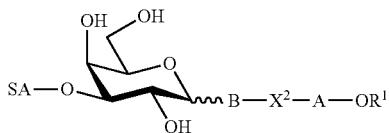
29

When m is 0, the D-galactopyranosyl unit is directly coupled to group A through an interglycosidic linkage. Preferably, the interglycosidic linkage is a 1-4 linkage thus forming compounds of general formula 1'-3B and salts thereof



wherein R^3 is fucosyl or H. More preferably the interglycosidic linkage between the galactose and the glucose portion is β thus giving rise to a 3'-O-sialyl-lactose derivative. In an even more preferable embodiment aglycon —OR¹ is also in β orientation.

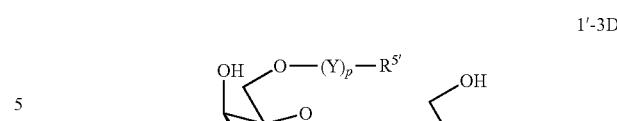
When m is 1, the structural element X¹, as carbohydrate linker, means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure. The monosaccharide building units of the carbohydrate linker can be any naturally occurring 5-, 6- or 9-carbon containing sugar derivatives, with the most frequently occurring units being glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Preferably, linker X¹ is represented by the formula —B—X²— thus forming compounds of general formula 1'-3C and salts thereof



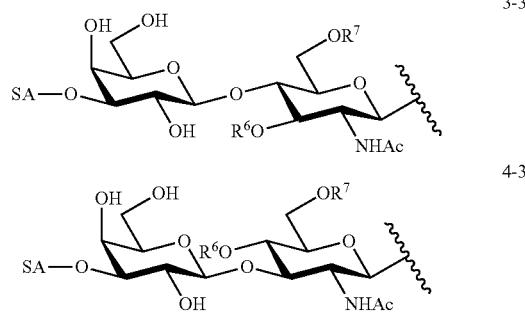
wherein group B is an N-acetyl-glucosaminopyranosyl unit optionally substituted with fucosyl and/or sialyl, and linker X² means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure having the monosaccharide building units selected from glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Group —OR¹ is linked to the anomeric carbon (C₁) atom of the D-glucopyranosyl ring, preferably in β orientation. In preference, the sialyl-galactosyl group is attached to group B through 1-3 or 1-4 interglycosidic linkage forming thus a sialyl-lacto-N-biosyl or sialyl-N-acetyl-lactosaminyl terminal trisaccharide moieties, respectively. In a further favoured method, a fucosyl substituent may be coupled to the 3-OH or 4-OH group of unit B and/or to the 3-OH group of unit A, and/or sialyl may be connected to 6-OH of unit B.

In a more preferred embodiment, group X² in compounds of general formula 1'-3C and salts thereof is galactose optionally substituted with sialyl or oligosaccharide representing a linear or a branched structure having the saccharide building units selected from N-acetyl-lactosamine, lacto-N-biose, fucose and sialic acid, forming thus human milk oligosaccharide derivatives represented by general formula 1'-3D and salts thereof

30



wherein R¹ is a group removable by hydrogenolysis, R³ is H or fucosyl unit, Y is independently N-acetyl-lactosaminyl group optionally substituted with a sialyl and/or fucosyl residue, integer p is independently 0, 1 or 2, R⁴ is selected from the groups characterized by general formulae 3-3 and 4-3,



wherein R⁶ is H or fucosyl residue, R⁷H or α -sialyl moiety, SA is α -sialyl moiety and R^{5'} is selected from H, α -sialyl moiety, group of general formula 3-3 and group of general formula 4-3.

According to a further preferred embodiment, the compound of general formula 1'-3D and salts thereof as defined above is characterized by its linkages and attached moieties, wherein

an N-acetyl-lactosaminyl group in group Y (p=2), when attached to another N-acetyl-lactosaminyl group, is coupled with 1-3 interglycosidic linkage,
the group of general formula 3-3, when attached to Y (p=1,
2), is coupled with 1-3 interglycosidic linkage,
the group of general formula 4-3, when attached to Y (p=1,
2), is coupled with 1-3 interglycosidic linkage,
the fucosyl residue if attached to a N-acetyl-lactosaminyl group present in Y is linked to the N-acetyl-glucosamine of the N-acetyl-lactosaminyl group with 1-3 interglycosidic linkage,
the α -sialyl residue if attached to a N-acetyl-lactosaminyl present in Y is linked to the galactose of the N-acetyl-lactosaminyl group with 2-6 interglycosidic linkage.

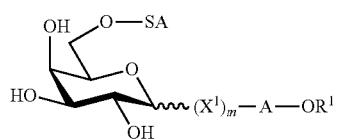
In a further aspect, the compound of general formula 1'-3B, 1'-3C or 1'-3D and salts thereof as defined above is selected from the group consisting of the R¹-glycosides of lactose, lacto-N-neotetraose, para-lacto-N-hexaose, para-lacto-N-neohexaose, lacto-N-neohexaose, para-lacto-N-octaose and lacto-N-neooctaose, lacto-N-tetraose, lacto-N-hexaose, lacto-N-octaose, iso-lacto-N-octaose, lacto-N-decaose and lacto-N-neodecaose optionally substituted with one or more sialyl and/or fucosyl residue and having sialyl substituent in 3-OH of a terminal galactosyl residue, and salts thereof. Preferably, the sialyl substituent(s) is/are N-acetyl-neuraminy group(s).

Particularly preferably, the compound of general formula 1'-3B, 1'-3C or 1'-3D and salts thereof as defined above is

31

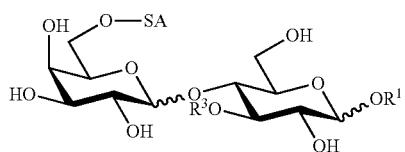
selected from the group consisting of R¹-glycosides of Neu5Aca2-3Galβ1-4Glc (3'-O—(N-acetyl-neuraminosyl)-lactose), Neu5Aca2-3Galβ1-4 (Fucα1-3)Glc (3-O-fucosyl-3'-O—(N-acetyl-neuraminosyl)-lactose), Neu5Aca2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc (LST a), Neu5Aca2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Neu5Aca2-3Galβ1-3 (Fucα1-4)GlcNAcβ1-3Galβ1-4Glc (FLST a), Neu5Aca2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc, Neu5Aca2-3Galβ1-3GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc, Neu5Aca2-3Galβ1-3 (Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc, Neu5Aca2-3Galβ1-3 (Neu5Aca2-6)GlcNAcβ1-3Galβ1-4Glc (DSLNT), Neu5Aca2-3Galβ1-3 (Neu5Aca2-6) (Fucα1-4)GlcNAcβ1-3Galβ1-4Glc (FDLNT I), Neu5Aca2-3Galβ1-3 (Neu5Aca2-6)GlcNAcβ1-3Galβ1-4 (Fucα1-3)Glc (FDLNT II), Neu5Aca2-3Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc, Neu5Aca2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc or salts thereof. The R¹-glycosides may be alpha- or beta-anomers. Preferably, said R¹-glycosides are the beta-anomers.

In another embodiment within the group of compounds of general formula 1A' and salts thereof the present invention relates to α-2-6-sialylated compounds of general formula 1'-6A and salts thereof.



wherein A is a D-glucopyranosyl unit optionally substituted with fucosyl, X¹ represents a carbohydrate linker, and integer m is 0 or 1. Group —OR¹ is linked to the anomeric carbon (C₁) atom of the D-glucopyranosyl ring, preferably in β orientation.

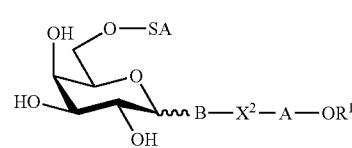
When m is 0, the D-galactopyranosyl unit is directly coupled to group A through an interglycosidic linkage. Preferably, the interglycosidic linkage is a 1-4 linkage thus forming compounds of general formula 1'-6B and salts thereof



wherein R³ is fucosyl or H. More preferably the interglycosidic linkage between the galactose and the glucose portion is β thus giving rise to a 3'-O-sialyl-lactose derivative. In an even more preferable embodiment aglycon—OR¹ is also in β orientation.

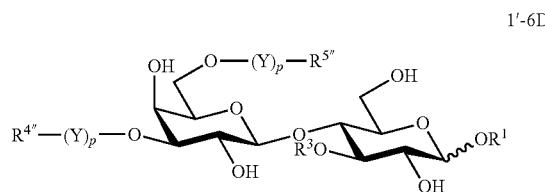
When m is 1, the structural element X¹, as carbohydrate linker, means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure. The monosaccharide building units of the carbohydrate linker can be any naturally occurring 5-, 6- or 9-carbon containing sugar derivatives, with the most frequently occurring units being glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Preferably, linker X¹ is represented by the formula —B—X²— thus forming compounds of general formula 1'-6C and salts thereof.

32

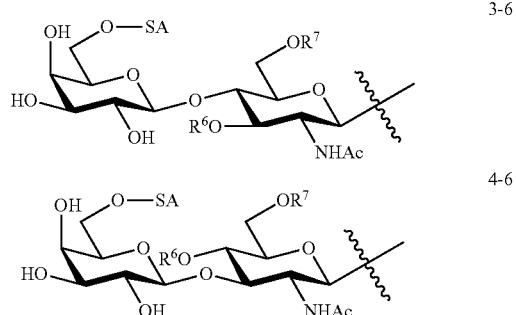


wherein group B is an N-acetyl-glucosaminopyranosyl unit optionally substituted with fucosyl and/or sialyl, and linker X² means a mono-, di-, tri-, tetra-, penta- or oligosaccharide representing a linear or a branched structure having the monosaccharide building units selected from glucose, N-acetyl-glucosamine, galactose, fucose and sialic acid. Group —OR¹ is linked to the anomeric carbon (C₁) atom of the D-glucopyranosyl ring, preferably in β orientation. In preference, the sialyl-galactosyl group is attached to group B through 1-3 or 1-4 interglycosidic linkage forming thus a sialyl-lacto-N-biosyl or sialyl-N-acetyl-lactosaminyl terminal trisaccharide moieties, respectively. In a further favoured method, a fucosyl substituent may be coupled to the 3-OH or 4-OH group of unit B and/or to the 3-OH group of unit A, and/or sialyl may be connected to 6-OH of unit B.

In a more preferred embodiment, group X² in compounds of general formula 1'-6C and salts thereof is galactose optionally substituted with sialyl or oligosaccharide representing a linear or a branched structure having the saccharide building units selected from N-acetyl-lactosamine, lacto-N-biose, fucose and sialic acid, forming thus human milk oligosaccharide derivatives represented by general formula 1'-6D and salts thereof



wherein R¹ is a group removable by hydrogenolysis, R³ is H or fucosyl unit, Y is independently N-acetyl-lactosaminyl group optionally substituted with a sialyl and/or fucosyl residue, integer p is independently 0, 1 or 2, R^{4"} is selected from the groups characterized by general formulae 3-6 and 4-6,



wherein R⁶ is H or fucosyl residue, R⁷H or α-sialyl moiety, SA is α-sialyl moiety and R^{5"} is selected from H, α-sialyl moiety, group of general formula 3-6 and group of general formula 4-6.

According to a further preferred method, the compound of general formula 1'-6D and salts thereof as defined above is characterized by its linkages and attached moieties, wherein an N-acetyl-lactosaminyl group in group Y (p=2), when attached to another N-acetyl-lactosaminyl group, is coupled with 1-3 interglycosidic linkage, the group of general formula 3-6, when attached to Y (p=1, 2), is coupled with 1-3 interglycosidic linkage, the group of general formula 4-6, when attached to Y (p=1, 2), is coupled with 1-3 interglycosidic linkage, the fucosyl residue if attached to a N-acetyl-lactosaminyl group present in Y is linked to the N-acetyl-glucosamine of the N-acetyl-lactosaminyl group with 1-3 interglycosidic linkage, the α-sialyl residue if attached to a N-acetyl-lactosaminyl present in Y is linked to the galactose of the N-acetyl-lactosaminyl group with 2-6 interglycosidic linkage.

In a further aspect, the compound of general formula 1'-6B, 1'-6C or 1'-6D and salts thereof as defined above represents the R¹-glycosides of lactose, lacto-N-neotetraose, para-lacto-N-hexaose, para-lacto-N-neohexaose, lacto-N-neohexaose, para-lacto-N-octaose and lacto-N-neooctaose, lacto-N-tetraose, lacto-N-hexaose, lacto-N-octaose, iso-lacto-N-octaose, lacto-N-decaose and lacto-N-neodecaose optionally substituted with one or more sialyl and/or fucosyl residue and having sialyl substituent in 6-OH of a terminal galactosyl residue, and salts thereof. Preferably, the sialyl substituent(s) is/are N-acetyl neuraminyl group(s).

Particularly preferably, the compound of general formula 1'-6B, 1'-6C or 1'-6D and salts thereof as defined above is selected from the group of R¹-glycosides of Neu5Acα2-6Galβ1-4Glc (6'-O-(N-acetyl-neuraminosyl)-lactose), Neu5Acα2-6Galβ1-4(Fucα1-3)Glc, Neu5Acα2-6Galβ1-3GlcNAcβ1-3Galβ1-4Glc, Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc (LST c), Neu5Acα2-6Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc, Neu5Acα2-6Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc, Neu5Acα2-3GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc, Neu5Acα2-6Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc, Neu5Acα2-6Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3Galβ1-4Glc, Neu5Acα2-6Galβ1-3(Neu5Acα2-6)(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc, Neu5Acα2-6Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3Galβ1-4Glc, Neu5Acα2-6Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3Galβ1-4Glc, Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc, Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc (FLST c), Neu5Acα2-6Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc, Neu5Acα2-6Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc or salts thereof. The R¹-glycosides may be alpha- or beta-anomers. Preferably, said R¹-glycosides are the beta-anomers.

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not to be limiting thereof.

EXPERIMENTAL

1. Synthesis of Sialyl Acceptors

A) General procedure: lactose (5 g, 14.6 mmol) and TsOH.H₂O (0.2 g, 1.05 mmol) were added in one portion to a mixture of DMF (20 ml) and benzaldehyde dimethyl acetal (5.5 ml, 35.4 mmol, 2.4 eq.) at room temperature. The reaction mixture was stirred strongly at 70° C. under exclusion of humidity for 1 hour. After cooling triethyl amine (0.15 ml) was added then the volatile components (MeOH, triethyl amine, remaining benzaldehyde dimethyl acetal) were removed in vacuo. To the reaction mixture the benzyl bromide derivative (1.5 eq.)—predissolved in 5-10 ml of DMF, if the

reagent is a solid—was added and the mixture was cooled to 0° C. for 20 min. Still under cooling NaH (0.8 g of a 55% dispersion on mineral oil, 1.3 eq.) was added in one portion and the mixture was stirred under cooling until the hydrogen formation stopped then at room temperature for 2-3 hours. Methanol (2 ml) was added carefully and the reaction was stirred for a further 5 min. The reaction mixture was portioned between 100 ml of DCM and 100 ml of water and extracted. The water layer was back-extracted twice with 100 ml of DCM. The combined organic phases were evaporated, the residue was dissolved in 100 ml of acetonitrile and extracted with 100 ml of hexane. The acetonitrile was distilled off and the residue was taken up in isopropanol (10 ml) and isopropyl ether (50 ml) at 50° C. The clear solution was cooled to -20° C. for overnight. The crystals obtained were filtered off and washed twice with TBME and dried. Recrystallization may be carried out from a mixture of TBME (~50 ml) and ethanol (~20 ml).

4-Chlorobenzyl 4',6'-O-benzylidene-β-lactoside

Yield: 1.71 g

4-Methylbenzyl 4',6'-O-benzylidene-β-lactoside

Yield: 3.20 g

3-Phenylbenzyl 4',6'-O-benzylidene-β-lactoside

Yield: 2.70 g

2-Naphthylmethyl 4',6'-O-benzylidene-β-lactoside

Yield: 1.77 g

B) To a mixture of one of the above benzylidene acetals (500 mg) in methanol (10 ml) and water (0.5 ml) TFA was added at room temperature and the reaction mixture was stirred for 2-4 hours under exclusion of humidity then evaporated. The remaining material was co-evaporated with ethanol 3-4 times giving a crude solid, which, after drying, may be recrystallized from a mixture of methanol (~10-35 ml) and water (~0-2 mL).

4-Chlorobenzyl β-lactoside

Yield: 333 mg

¹³C-NMR (75.1 MHz, D₂O): δ=135.25, 133.67, 130.30, 128.70, 103.00, 101.13, 78.39, 75.44, 74.89, 74.49, 72.88, 72.58, 71.03, 70.83, 68.62, 61.11, 60.13.

4-Methylbenzyl β-lactoside

Yield: 439 mg

¹³C-NMR (75.1 MHz, D₂O): δ=138.91, 133.50, 129.37, 129.07, 103.01, 100.96, 78.43, 75.44, 74.87, 74.52, 72.90, 72.59, 71.47, 71.03, 68.63, 61.11, 60.17, 20.34.

3-Phenylbenzyl β-lactoside

Yield: 438 mg

¹³C-NMR (75.1 MHz, d₆-DMSO/d₄-MeOH/D₂O 8:1:1): δ=140.29, 140.24, 138.88, 129.13, 129.02, 127.66, 126.88, 126.83, 126.03, 125.90, 103.95, 102.03, 80.76, 75.65, 75.07, 75.00, 73.34, 73.28, 70.66, 69.81, 68.27, 60.56.

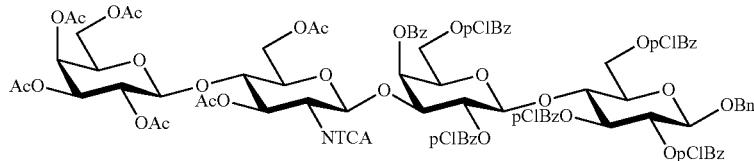
2-Naphthylmethyl β-lactoside

Yield: 378 mg

35

¹³C-NMR (75.1 MHz, D₂O/d₆-DMSO): δ=134.96, 133.24, 133.12, 128.59, 128.31, 128.08, 127.46, 126.98, 126.90, 126.79, 103.26, 101.59, 78.89, 75.62, 75.09, 74.81, 73.14, 72.81, 71.33, 71.14, 68.75, 61.22, 60.39.

C)



36

HCl-gas and concentrated to dryness. The resulting crude brown glass was then acetylated with pyridine (150 ml) and acetic anhydride (150 ml) at rt. for 1 d. The solution was concentrated, the syrup was dissolved in CH₂Cl₂, the organic phase was extracted with 1M HCl-solution and then with sat.

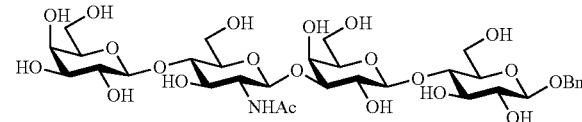
15

10 g (8.13 mmol) of benzyl 2,3,6,2',6'-penta-O-(4-chlorobenzoyl)-4'-O-benzoyl-β-lactoside and 10 g (1.6 equiv.) of methyl N-trichloroacetyl-3,6,2',3',4'6'-hexa-O-acetyl-1-thioglycosaminide were dissolved in 35 ml of dry CHCl₃ under argon. To this solution 3.7 g of NIS and 490 mg of AgOTF were added at rt, and the stirring was continued for approx. 20 min. Triethyl amine (5 ml) was added to the slurry, diluted with CH₂Cl₂ (500 ml) and then extracted 2x with sodium thiosulphate solution (10%), the organic phase was separated, dried with MgSO₄, filtered, concentrated, and the syrup was chromatographed on a column of silica-gel, using a gradient of CH₂Cl₂: acetone 98:2→95:5. Yield: 12.7 g, 80%. MS (ESP): 1972.1 [M+Na]⁺, 1988.1 [M+K]⁺, 1948.2 [M-H]⁻, 1984.0 [M+Cl]⁻. ¹³C NMR (CDCl₃) δ: 101.2, 100.8, 100.4, 99.2 (anomeric carbons).

20

NaHCO₃-solution, dried with MgSO₄, filtered and concentrated to yield 43 g of brown foam, which was subjected to column chromatography using CH₂Cl₂:acetone 8:2 as eluent. ¹³C NMR (CDCl₃) δ: 101.2, 100.8, 100.4, 99.2 (anomeric carbons).

25



30

140 g (107.5 mmol) of the peracetylated tetrasaccharide prepared above was dissolved in 1.5 L of MeOH, NaOMe-solution (1M) was added until pH 10, and the mixture was stirred at 50° C. overnight. The product crystallized from the reaction mixture. The mixture is allowed to cool to rt., then it was chilled, filtered, the filtrate was washed with cold EtOH, then dried to yield 69 g of benzyl β-LNnT as a white powder (86.5 mmol, 80%). ¹³C NMR (D₂O) δ: 105.6, 105.5, 105.4, 103.6 (anomeric carbons). Mp. 284-286° C.

40 D) General Procedure for the Preparation of a Neutral HMO Benzyl/Substituted Benzyl Glycoside

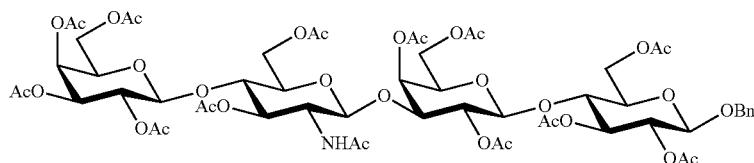
A selected neutral HMO (1 equiv.) was dissolved/suspended in 1-10 volumes (g/mL) of DMF, DMSO or a mixture thereof. The reaction mixture was cooled to 0° C. and benzyl bromide/substituted benzyl bromide (1.2-1.4 equiv.) was added. A strong base such as sodium hydride, potassium hydride, calcium hydride, potassium t-butoxide, sodium t-butoxide (1.2-1.4 equiv) was added at 0-40° C. and the reaction mixture was stirred for 6-24 hours at 0-60° C. Subsequently, water was added to quench the excess of base and the reaction mixture was stirred at RT for 30 minutes. The resulting reaction mixture was concentrated and purified in reverse phase

45

50

10 g (5.1 mmol) of tetrasaccharide prepared above was dissolved in MeOH (110 ml) and a solution of NaOMe (1 M in MeOH) was added until pH 10 was attained. The solution was stirred at 40° C. for 5 h, then was neutralized by addition of Amberlite IR 120H⁺ resin, the resin was filtered off, and the filtrate was evaporated to dryness. The residue was dissolved in warm DMF (10 ml) and added dropwise to ³Pr₂O (150 ml) and the suspension was stirred for an additional 3 h. The precipitate was filtered off, washed with ³Pr₂O (2×20 ml) and dried to yield 4.2 g of product as off-white powder (91%). MS (ESP): 900.1 [M-H]⁻. ¹³C NMR (D₂O) δ: 105.6, 105.5, 104.2, 103.7 (anomeric carbons).

55



35 g of a compound of the tetrasaccharide prepared above was dissolved in 110 ml of MeOH and 110 ml of aqueous KOH (7.5 g) solution and the mixture was stirred at rt. for id. The mixture was then chilled with an ice-bath, neutralized by

65 chromatography, silica gel chromatography, ion-exchange chromatography, size-exclusion chromatography, etc. or crystallized giving rise to the desired benzylated/substituted benzylated neutral HMO compound in 70-80% yields.

1-O- β -(4-methylbenzyl)-LNnT

¹H NMR (D₂O): 7.3 (dd, 4H), 4.88 (d, 1H), 4.7 (m), 4.54 (d, 1H), 4.48 (d, 1H), 4.42 (d, 1H), 4.34 (d), 4.0-3.5 (m), 3.34 (dd, 1H).

¹³C NMR (D₂O): 184.2, 177.6, 173.7, 141.5, 136.1, 131.9, 131.4, 105.6, 105.5, 105.4, 103.6, 93.2, 84.7, 81.5, 81.0, 80.8, 78.0, 77.6, 77.4, 77.1, 75.5, 75.2, 74.8, 74.0, 73.6, 72.9, 63.7, 62.8, 58.9, 56.4, 25.9, 22.9.

1-O- β -(4-chlorobenzyl)-LNnT

¹H NMR (D₂O): 7.4 (s, 4H), 4.9 (d, 1H), 4.72 (m), 4.52 (d, 1H), 4.8 (d, 1H), 4.42 (d, 1H), 4.16 (d, 1H), 4.0-3.52 (m).

¹³C NMR (D₂O): 138.9, 177.6, 138.3, 137.9, 136.2, 131.3, 105.6, 105.5, 105.4, 103.7, 93.2, 86.1, 84.7, 81.5, 81.0, 80.8, 78.0, 77.5, 77.4, 77.2, 77.1, 75.5, 75.2, 74.9, 63.7, 58.9, 57.8, 56.4, 24.8.

1-O- β -benzyl-LNT

¹H-NMR (D₂O, 400 MHz) δ 2.03 (s, 3H, CH₃CONH), 3.35 (dd, 1H, J=8.1 8.5 Hz, H-2), 3.49 (m, 1H, H-5"), 3.53 (m, H-2"), 3.65 (m, 1H, H-3"), 3.57 (dd, 1H, J=8.1 9.0 Hz, H-4"), 3.58 (m, 1H, H-5), 3.59 (dd, 1H, J=7.7 10.0 Hz, H-2'), 3.62 (m, 1H, H-3), 3.63 (m, 1H, H-4), 3.71 (m, 1H, H-5'), 3.71 (m, 1H, H-5"), 3.73 (dd, 1H, J=3.3 10.0 Hz, H-3'), 3.76 (m, 2H, H-6ab"), 3.76 (m, 2H, H-6ab'), 3.80 (m, 1H, H-6a"), 3.80 (dd, 1H, J=5.0 12.2 Hz, H-6a), 3.82 (dd, 1H, J=8.1 10.5 Hz, H-3"), 3.90 (m, 1H, H-6b"), 3.90 (dd, 1H, J=8.4 10.5 Hz, H-2"), 3.92 (d, 1H, J=3.3 Hz, H-4"), 3.98 (dd, 1H, J=1.6 12.2 Hz, H-6b), 4.15 (d, 1H, J=3.3 Hz, H-4'), 4.44 (d, 1H, J=7.7 Hz, H-1'), 4.45 (d, 1H, J=7.7 Hz, H-1"), 4.56 (d, 1H, J=8.1 Hz, H-1), 4.73 (d, 1H, J=8.4 Hz, H-1'), 4.76 (d, 1H, J=11.7 Hz, CH₂Ph), 4.94 (d, 1H, J=11.7 Hz, CH₂Ph), 7.40-7.50 (m, 5H, Ph).

¹³C-NMR (D₂O, 100 MHz) δ 24.9 (CH₃CONH), 57.4 (C-2"), 62.8 (C-6), 63.2 (C-6"), 63.7 (C-6""), 63.7 (C-6'), 71.0 (C-4'), 71.2 (C-4""), 71.3 (C-4"), 72.7 (C-2'), 73.4 (C-2"), 74.2 (CH₂Ph), 75.2 (C-3"), 75.5 (C-2), 77.1 (C-3), 77.5 (C-5'), 77.6 (C-5""), 77.9 (C-5), 78.0 (C-5"), 81.1 (C-4), 84.7 (C-3'), 84.8 (C-3"), 103.7 (C-1), 105.3 (C-1"), 105.6 (C-1'), 106.2 (C-1"), 131.1 (Ph), 131.4 (2C, Ph), 131.5 (2C, Ph), 139.2 (Ph), 177.7 (CH₃CONH).

M.p. 245° C. (dec.). [α]_D²²=-10.3 (c=1, H₂O).

1-O- β -(4-methylbenzyl)-LNT

¹H-NMR (D₂O, 300 MHz) δ 1.97 (s, 3H), 2.29 (s, 3H), 3.27 (dd, 1H, J=8.1 8.5 Hz), 3.39-3.87 (m, 21H), 3.92 (dd, 1H, J=1.8 12.3 Hz), 4.09 (d, 1H, J=3.3 Hz), 4.37 (d, 1H, J=8.1 Hz), 4.38 (d, 1H, J=7.8 Hz), 4.47 (d, 1H, J=8.1 Hz), 4.65 (d, 1H, J=11.7 Hz), 4.67 (d, 1H, J=8.1 Hz), 4.83 (d, 1H, J=11.7 Hz), 7.22 (d, 2H, J=8.1 Hz), 7.30 (d, 2H, J=8.1 Hz).

¹³C-NMR (D₂O, 85.4 MHz) δ 23.1, 25.0, 57.7, 62.8, 63.2, 63.7, 63.8, 71.0, 71.1, 71.3, 72.7, 73.4, 74.1, 75.2, 75.5, 77.1, 77.5, 77.6, 77.9, 78.0, 81.1, 84.7, 84.8, 103.6, 105.3, 105.7, 106.2, 131.7 (2C), 132.0 (2C), 136.2, 141.5, 177.7.

2. Trans-Sialylation Reactions

General procedure: a solution of 2-O-(p-nitrophenyl)- α -D-sialoside (25 mmol) and the appropriate sialyl acceptor (35 mmol) in degassed incubation buffer (1.0 M, 100 mM Tris/HCl, pH 7.5, 50 mg BSA, 0.02% NaNO₃) was incubated with recombinant transsialidase from *T. cruzi* (80 μ L, 1.3 mg/ml) at 23° C. for 24 h. The reaction was monitored by TLC (butanol/acetic acid/water 5:2:2). After completion, the enzyme was

denatured and centrifuged before the supernatant is lyophilized. The dry residue was dissolved in water and purified by biogel chromatography (P-2 Biogel, 16×900 mm) with water or by reverse phase chromatography. The yields vary between 45-85%.

4-Chlorobenzyl 3'-O—(N-acetyl-neuraminosyl)- β -lactoside

¹⁰ ¹H-NMR (500 MHz, D₂O): δ [ppm]=7.46-7.42 (m, 4H, H_{(a/b)arom}); 4.91 (d, 1H, CH₂a-Bn); 4.74 (d, 1H, CH_bBn); 4.55-4.52 (m, 2H, H-1/H-1'); 4.11 (dd, 1H, H-3'); 2.76 (dd, 1H, H-3"_{eq}); 2.04 (s, 3H, COCH₃); 1.81 (dd, 1H, H-3"_{ax}). J_{(a,b)-Bn}=11.8; J_{2',3'}=9.9; J_{3',4'}=2.9; J_{3''ax,3''eq}=12.4; J_{3''ax,4''}=12.1; J_{3''eq,4''}=4.5 Hz.
¹³C-NMR (126 MHz, D₂O): δ [ppm]=135.2, 133.5 (quart. C_{arom}); 130.2 (CH_{a-arom}); 128.6 (CH_{b-arom}); 102.6 (C-1'); 101.1 (C-1); 51.7 (C-5"); 39.6 (C-3"); 22.0 (COCH₃).

²⁰ 4-Methylbenzyl 3'-O—(N-acetyl-neuraminosyl)- β -lactoside

¹H-NMR (500 MHz, D₂O): δ [ppm]=7.36 (d, 2H, H-a_{arom}); 7.28 (d, 2H, H-b_{arom}); 4.89 (d, 1H, CH₂a-Bn); 4.72 (d, 1H, CH₂b-Bn); 4.54-4.52 (m, 2H, H-1/H-1'); 4.12 (dd, 1H, H-3'); 2.76 (dd, 1H, H-3"_{eq}); 2.35 (s, 3H, CH₃-Tol); 2.04 (s, 3H, COCH₃); 1.81 (dd, 1H, H-3"_{ax}). J_{(a,b)arom}=7.9; J_{(a,b)-Bn}=11.5; J_{2',3'}=9.9; J_{3',4'}=3.0; J_{3''ax,3''eq}=12.5; J_{3'',4''}=12.2; J_{3''eq,4''}=4.6 Hz.

¹³C-NMR (126 MHz, D₂O): δ [ppm]=138.8, 133.4 (quart. C_{arom}); 129.3 (CH_{a-arom}); 129.0 (CH_{b-arom}); 102.6 (C-1'); 100.9 (C-1); 99.8 (C-2"); 51.7 (C-5"); 39.6 (C-3"); 22.0 (COCH₃); 20.2 (CH₃-Tol).

2-Naphthylmethyl 3'-O—(N-acetyl-neuraminosyl)- β -lactoside

¹H-NMR (500 MHz, D₂O): δ [ppm]=7.99-7.97 (m, 4H, H-arom); 7.62-7.59 (m, 3H, H-arom); 5.10 (d, 1H, CH₂a-Bn); 4.95 (d, 1H, CH₂b-Bn); 4.60 (d, 1H, H-1); 4.53 (d, 1H, H-1'); 4.12 (dd, 1H, H-3'); 2.77 (dd, 1H, H-3"_{eq}); 2.04 (s, 3H, COCH₃); 1.81 (dd, 1H, H-3"_{ax}). J_{(a,b)-Bn}=11.9; J_{1',2'}=8.0; J_{1',2'}=7.9; J_{2',3'}=9.9; J_{3',4'}=3.0; J_{3''ax,3''eq}=12.5; J_{3'',4''}=12.1; J_{3''eq,4''}=4.6 Hz.

¹³C-NMR (126 MHz, D₂O): δ [ppm]=128.3, 127.9, 127.7, 127.6, 126.6, 126.5 (CH_{arom}); 102.6 (C-1'); 101.1 (C-1); 51.7 (C-5"); 22.0 (COCH₃).

3-Phenylbenzyl 3'-O—(N-acetyl-neuraminosyl)- β -lactoside

¹H-NMR (400 MHz, D₂O): δ [ppm]=7.75-7.44 (m, 9H, H-arom); 4.99 (d, 1H, CH₂a-Bn); 4.82 (d, 1H, CH₂b-Bn); 4.57 (d, 1H, H-1); 4.53 (d, 1H, H-1'); 4.13 (dd, 1H, H-3'); 2.78 (dd, 1H, H-3"_{eq}); 2.05 (s, 3H, COCH₃); 1.82 (dd, 1H, H-3"_{ax}). J_{(a,b)-Bn}=11.8; J_{1',2'}=8.0; J_{1',2'}=7.9; J_{2',3'}=9.9; J_{3',4'}=3.1; J_{3''ax,3''eq}=12.5; J_{3'',4''}=12.0; J_{3''eq,4''}=4.6 Hz.

¹³C-NMR (100 MHz, D₂O): δ [ppm]=140.9, 140.3, 137.5 (quart. C_{arom}); 129.4, 129.2, 127.9, 127.8, 127.1, 127.0, 126.9, (CH_{arom}); 102.7 (C-1'); 101.2 (C-1); 99.6 (C-2"); 51.8 (C-5"); 39.7 (C-3"); 22.1 (COCH₃).

Benzyl 3"-O—(N-acetyl-neuraminosyl)- β -LNnT

¹H-NMR (400 MHz, D₂O): see FIG. 1.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 11

<210> SEQ ID NO 1
<211> LENGTH: 393
<212> TYPE: PRT
<213> ORGANISM: *Bifidobacterium longum* subsp. *infantis* ATCC 15697
<220> FEATURE:
<221> NAME/KEY: source
<222> LOCATION: 1..393
<223> OTHER INFORMATION: /mol_type="protein"
/organism="*Bifidobacterium longum* subsp. *infantis* ATCC 15697"

<400> SEQUENCE: 1

Met Thr Glu Asn Gly Met Met Asn Thr Asn Asn Thr Val Cys Gly Ala
1 5 10 15

Asn His Asp Gly Ala Met Ser Leu Ala Ala Pro Gly Asp Tyr Gly Val
20 25 30

Ala Cys Tyr Arg Ile Pro Ala Leu Ala Glu Ala Pro Asn Gly Trp Ile
35 40 45

Leu Ala Ala Phe Asp Ala Arg Pro His Asn Cys Gln Asp Ala Pro Gln
50 55 60

Ala Asn Ser Ile Val Gln Arg Ile Ser Lys Asp Gly Gly Arg Ser Phe
65 70 75 80

Glu Pro Gln His Val Val Ala Ala Gly His Asp Gly Val Asp Lys Tyr
85 90 95

Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp Arg Gln Thr Gly Glu Val
100 105 110

Phe Leu Phe Phe Val Lys Ser Tyr Asp Ala Gly Phe Gly Thr Ser Gln
115 120 125

Ala Gly Val Asp Pro Ser Ala Arg Glu Val Leu Gln Ala Ala Val Thr
130 135 140

Ser Ser Ile Asp Asn Gly Val Thr Trp Ser Glu Pro Arg Ile Ile Thr
145 150 155 160

Ala Asp Ile Thr Asn Ser Glu Ser Trp Ile Ser Arg Phe Ala Ser Ser
165 170 175

Gly Ala Gly Ile Gln Leu Thr Tyr Gly Glu His Ala Gly Arg Leu Ile
180 185 190

Gln Gln Tyr Thr Ile Lys Glu Leu Asp Gly Arg Tyr Arg Ala Val Ser
195 200 205

Val Phe Ser Asp Asp His Gly Ala Thr Trp His Ala Gly Thr Pro Val
210 215 220

Gly Asp His Met Asp Glu Asn Lys Val Val Glu Leu Ser Asp Gly Arg
225 230 235 240

Val Met Leu Asn Ser Arg Ser Ser Asp Gly Asn Gly Cys Arg Tyr Val
245 250 255

Ala Ile Ser Arg Asp Gly Gly Ala Thr Tyr Gly Pro Val Ile Arg Glu
260 265 270

Thr Gln Leu Pro Asp Pro Glu Asn Asn Ala Gln Ile Ala Arg Ala Phe
275 280 285

Pro Asp Ala Pro Glu Gly Ser Ala Gln Ala Lys Val Leu Leu Tyr Ser
290 295 300

Ser Ser Ser Pro Ser Asp Arg Ile Asp Gly Leu Val Arg Val Ser Ile
305 310 315 320

Asp Asp Gly Lys Thr Trp Ser Ala Gly Arg Arg Phe Thr Thr Gly Pro
325 330 335

Met Ala Tyr Ser Val Ile Ala Ala Leu Ser His Lys Ala Gly Gly

-continued

340	345	350
Tyr Gly Leu Leu Tyr Glu Gly Asp Asn Asn Asn Ile Met Tyr Thr Arg		
355	360	365
Ile Ser Leu Asp Trp Leu Asn Gly Gln Leu Asn Val Asp Gly Ile Gly		
370	375	380
Gly Phe Pro Leu Ser Gly Glu Gly Gly		
385	390	

<210> SEQ ID NO 2
<211> LENGTH: 760
<212> TYPE: PRT
<213> ORGANISM: *Bifidobacterium longum* subsp. *infantis* ATCC 15697
<220> FEATURE:
<221> NAME/KEY: source
<222> LOCATION: 1..760
<223> OTHER INFORMATION: /mol_type="protein"
/organism="*Bifidobacterium longum* subsp. *infantis* ATCC 15697"

<400> SEQUENCE: 2

Met Ala Ala Ser Asn Pro Ile Ser Trp Ser Gln Arg Thr Phe Pro Ser			
1	5	10	15
Pro Glu Gly Thr Ile Ala Cys Arg Phe Arg Ala His Ala Asp Gly Arg			
20	25	30	
Ile Phe Asp Ala Val Asn Gly Ser Ala Asn Asp Ala Pro Leu Leu Ile			
35	40	45	
Cys Ala Ile Glu His Asp Ala Leu Arg Val Arg Ala Thr Thr Pro Arg			
50	55	60	
Gln His Val Asp Phe Asp Ile Glu Asp Thr Thr Gly Ile Ala Asp Gly			
65	70	75	80
Ala Met His Thr Phe Ala Leu Thr Phe Gly Glu Phe Gly Thr Arg Val			
85	90	95	
Tyr Leu Asp Gly Ser Gln Cys Phe Ser Gly Thr Ala Asn Leu Cys Pro			
100	105	110	
Thr Thr Leu Thr Gly Thr Glu Gly Ser Gly Gln Gly Ala Ile Arg Leu			
115	120	125	
Ala Gly Pro Ser Ile Asp Val Thr Asp Met Arg Leu His Ala Ile Pro			
130	135	140	
Leu Thr Ser Glu Ser Ile Ala Ala Leu Thr Pro Arg Pro Ala Pro Asp			
145	150	155	160
Ile Asp Phe Ala Ala Ala Gln Leu Ala Pro Arg Asp Val Arg Arg Val			
165	170	175	
Arg Thr Leu Arg Ser Gly Thr Ile Phe Met His Phe Arg Val Arg Gly			
180	185	190	
Pro Arg Gln Tyr Gly Thr Leu Leu Ala Ala Gly Glu Arg Gly Glu Glu			
195	200	205	
Arg Leu Ala Val Ser Ile Asp Asp Asn Gly Ile Thr Met Thr Ala Ala			
210	215	220	
Asp Gly Leu Tyr Glu Pro Ser Thr Tyr His Ala Arg Gly Ala Trp Asp			
225	230	235	240
Asp Gly Arg Trp His Asp Leu Ser Ile Arg Ser Ala Arg Gly Ala Ile			
245	250	255	
Asp Met Tyr Val Asp Gly Trp His Glu Leu His Gln Ala Gly Gln Val			
260	265	270	
Phe Phe Gly Asp Trp Pro Gln Leu Asp Glu Val Ala Ile Gly Gln Asn			
275	280	285	
Thr Glu Gly Val Arg Leu Met Gly Glu Val Arg Asn Gly Gly Val Phe			

-continued

290	295	300
Thr Thr Pro Leu Thr Asp Gly Ala Ile Arg Arg		
305	310	315
Leu Ser Asp Ala Pro		320
Ala Leu Thr Thr Ala Leu Phe Asp Lys Gly Tyr His	Gly Ser Val	
325	330	335
Ser Tyr Arg Ile Pro Ser Ile Ile Arg Thr Pro His	Gly Val Val Val	
340	345	350
Ala Gly Ala Asp Gln Arg Thr Ala Ile Ala Asn Asp	Ala Pro Asn His	
355	360	365
Ile Asn Phe Val Met Arg Arg Ser Leu Asp Gly Gly	Arg Thr Trp Leu	
370	375	380
Asp Met Gln Thr Val Ile Ala Asn Pro Gly Glu Gly	Val Asp Gly Ala	
385	390	395
Cys Thr Ile Asp Ser Cys Leu Val Cys Asp Glu Arg	Asn Gly Arg Leu	
405	410	415
Thr Val Leu Ile Asp Arg Phe Ala Gly Gly Val Gly	Leu Pro Asn Asn	
420	425	430
Thr Pro Gly Thr Gly Val Asp Arg His Gly Arg Pro	Cys Leu Tyr Asp	
435	440	445
Arg Ala Gly Thr Arg Tyr Val Leu Ala Asp Asp Gly	Thr Val Leu Asp	
450	455	460
Gly Gly Gly Glu Arg Thr Gly Tyr Arg Val Asp Ala	His Gly Asn Val	
465	470	475
480		
Thr His Glu Gly Arg Ala Ser Gly Asn Ile Tyr Leu	Lys Glu Gly Ala	
485	490	495
Asp Pro Asp Glu Ser Leu Leu Ile Glu Arg Thr Ser	Phe Ile Ile Glu	
500	505	510
Leu His Ser Asp Asp Asp Gly Glu Thr Trp Ser Thr	Pro Arg Asn Ile	
515	520	525
Asn His Met Ile Lys Glu Asp Trp Met His Phe Leu	Gly Val Ser Pro	
530	535	540
Gly Asn Gly Ile Gln Leu Gln Ala Ser Glu His Arg	Gly Arg Leu Leu	
545	550	555
560		
Val Pro Phe Tyr Cys Thr Gly Ala Ser Leu Lys His	Tyr Ser Gly Gly	
565	570	575
Ala Leu Ile Ser Asp Asp Gly Gly Asp Thr Trp Arg	Arg Gly Ser Met	
580	585	590
Ile Asn Asp Gly Arg Ile Val Asn Gly Thr Ala Val	Asp Pro Lys Asn	
595	600	605
Ile Arg Asp Asp Ala Thr Thr His Glu Ser Val Phe	Val Glu Arg	
610	615	620
Ala Asp Gly Thr Val Val Cys Phe Phe Arg Asn Gln	Asn His Ala Gly	
625	630	635
640		
Arg Ile Gly Val Ala Leu Ser His Asp Gly Gly	Glu Thr Trp Asp Asp	
645	650	655
Leu Tyr Phe Asp Lys Asp Val Pro Asp Ile Phe Cys	Gln Pro Asn Ala	
660	665	670
Val Ala Cys Ala Pro Arg Ser Asp Thr Met Val Phe	Ala Asn Ala Ser	
675	680	685
Gln Met Leu Pro Tyr Arg Gly Asn Gly Val Leu Arg	Leu Ser Leu Asp	
690	695	700
Gly Ala Arg Thr Trp Ala Ala His Arg Cys Ile Asn	Pro Tyr His Tyr	
705	710	715
720		

-continued

Gly Tyr Gln Cys Met Thr Met Leu Pro Asp Gly Glu Leu Gly Leu Leu
725 730 735

Trp Glu Arg Glu Thr Ala Gly Leu Tyr Phe Thr Thr Leu Pro Leu Ser
740 745 750

Val Phe Gly Ala Ala Glu Thr His
755 760

<210> SEQ ID NO 3
<211> LENGTH: 836
<212> TYPE: PRT
<213> ORGANISM: Bifidobacterium bifidum
<220> FEATURE:
<221> NAME/KEY: source
<222> LOCATION: 1..836
<223> OTHER INFORMATION: /mol_type="protein"
/organism="Bifidobacterium bifidum"

<400> SEQUENCE: 3

Met Val Arg Ser Thr Lys Pro Ser Leu Leu Arg Arg Leu Gly Ala Leu
1 5 10 15

Val Ala Ala Ala Met Leu Val Val Leu Pro Ala Gly Val Ser Thr
20 25 30

Ala Ser Ala Ala Ser Asp Asp Ala Asp Met Leu Thr Val Thr Met Thr
35 40 45

Arg Thr Asp Thr Leu Gly Asp Glu Val Tyr Val Gly Asp Thr Leu Thr
50 55 60

Tyr Ser Phe Thr Asn Thr Asn Asn Thr Ser Ser Ala Phe Thr Ala Phe
65 70 75 80

Pro Ala Glu Ser Asn Leu Ser Gly Val Leu Thr Thr Gly Thr Pro Asn
85 90 95

Cys Arg Tyr Glu Asn Leu Ala Gly Ala Ser Tyr Pro Cys Ser Thr
100 105 110

Ala Ser His Thr Ile Thr Ala Asp Asp Leu Thr Ala Gly Ser Phe Thr
115 120 125

Pro Arg Thr Val Trp Lys Ala Thr Ser Asp Arg Gly Gly Thr Gln Val
130 135 140

Leu Gln Asp Asn Ile Val Ser Thr Gly Asp Thr Val Thr Val Lys Glu
145 150 155 160

Gly Lys Arg Pro Asp Pro Ala Thr Ile Pro Thr Asp Arg Ala Asp Gly
165 170 175

Glu Ala Val Arg Leu Ala Thr Ala Arg Gln Asn Leu Gly Thr Glu Cys
180 185 190

Tyr Arg Ile Pro Ala Leu Ala Glu Ala Pro Asn Gly Trp Ile Leu Ala
195 200 205

Ala Phe Asp Gln Arg Pro Asn Thr Ala Met Ala Asn Gly Ser Gly Val
210 215 220

Lys Cys Trp Asp Ala Pro Gln Pro Asn Ser Ile Val Gln Arg Ile Ser
225 230 235 240

Lys Asp Gly Gly Lys Ser Trp Thr Pro Ile Gln Tyr Val Ala Gln Gly
245 250 255

Lys Asn Ala Pro Glu Arg Tyr Gly Tyr Ser Asp Pro Ser Tyr Val Val
260 265 270

Asp Glu Glu Thr Gly Glu Ile Phe Leu Phe Phe Val His Ser Tyr Asn
275 280 285

Lys Gly Phe Ala Asp Ser Gln Leu Gly Val Asp Glu Ser Asn Arg Asn
290 295 300

-continued

Val Leu His Ala Val Val Val Ser Ser Lys Asp Asn Gly Glu Thr Trp
305 310 315 320

Ser Lys Pro Arg Asp Ile Thr Ala Asp Ile Thr Lys Gly Tyr Glu Asn
325 330 335

Glu Trp Lys Ser Arg Phe Ala Thr Ser Gly Ala Gly Ile Gln Leu Lys
340 345 350

Tyr Gly Lys Tyr Lys Gly Arg Leu Ile Gln Gln Tyr Ala Val Gly Arg
355 360 365

Thr Thr Gly Ser Asn Ala Ala Val Ser Val Tyr Ser Asp Asp His Gly
370 375 380

Lys Thr Trp Gln Ala Gly Asn Pro Val Thr Gly Met Leu Met Asp Glu
385 390 395 400

Asn Lys Val Val Glu Leu Ser Asp Gly His Val Met Leu Asn Ser Arg
405 410 415

Pro Gly Asn Gly Ser Gly Tyr Arg Arg Val Ala Ile Ser Glu Asp Gly
420 425 430

Gly Val Asn Tyr Gly Thr Val Lys Asn Glu Thr Gln Leu Pro Asp Pro
435 440 445

Asn Asn Asn Ala His Ile Thr Arg Ala Phe Pro Asn Ala Pro Glu Gly
450 455 460

Ser Ala Lys Ala Lys Val Leu Leu Tyr Ser Ser Pro Arg Ala Asn Asn
465 470 475 480

Glu Gly Arg Ala Asn Gly Val Val Arg Ile Ser Leu Asp Asp Gly Thr
485 490 495

Thr Trp Ser Ser Gly Lys Leu Tyr Lys Ala Gly Ser Met Ala Tyr Ser
500 505 510

Val Ile Thr Ala Leu Ser Gly Ala Ala Gly Gly Tyr Gly Leu Leu
515 520 525

Tyr Glu Gly Ala Trp Val Thr Gly Gly Ile Asp Ser His Asp Ile
530 535 540

Met Tyr Thr His Ile Ser Met Asp Trp Leu Gly Tyr Leu Ser Ala Thr
545 550 555 560

Ala Asp Asp Val Thr Ala Ser Val Glu Glu Gly Ala Ser Thr Val Asp
565 570 575

Val Thr Val Pro Val Ser Asn Val Gly Ser Val Asp Tyr Thr Gly Val
580 585 590

Thr Val Thr Pro Ala Asp Leu Pro Thr Gly Trp Ser Ala Ser Pro Val
595 600 605

Asn Val Gly Ala Leu Ala Ser Gly Ala Ser Lys Asp Val Thr Val Thr
610 615 620

Val Asn Val Pro Ala Thr Ala Lys Lys Asp Asp Val Ala Lys Ile Val
625 630 635 640

Leu Arg Val Thr Gly Thr Ser Ala Ala Asn Ala Asp Ala Thr Thr Gly
645 650 655

Phe Asp Gly Ser Ile Thr Val Asn Val Thr Glu Lys Ser Glu Pro Asp
660 665 670

Pro Glu Pro Glu Pro Thr Ile Thr Gly Val Ser Ala Val Thr Ser Gln
675 680 685

Ala Gly Val Lys Val Gly Asp Val Phe Asp Ala Ser Lys Val Ser Val
690 695 700

Thr Ala Ala Met Ser Asp Gly Ser Ser Lys Ala Leu Ala Ala Gly Glu
705 710 715 720

US 9,102,966 B2

49**50**

-continued

Tyr Ser Leu Ser Ala Val Asp Ala Asp Gly Lys Ala Val Asp Leu Ala
725 730 735

Glu Pro Phe Ala Ala Ala Gly Val Val Thr Val Thr Val Ser Val Pro
740 745 750

Val Glu Gly Ala Asp Pro Leu Thr Ala Ser Phe Thr Ile Asp Val Ala
755 760 765

Glu Lys Ser Ala Asp Pro Glu Pro Lys Pro Glu Pro Glu Pro Lys Pro
770 775 780

Glu Pro Glu Lys Pro Ala Gly Pro Lys Val Asp Val Pro Thr Glu Lys
785 790 795 800

Pro Gly Leu Ser Lys Thr Gly Ala Ser Thr Ala Gly Met Ser Ile Val
805 810 815

Phe Val Leu Leu Ala Leu Ser Gly Val Ala Ala Leu Ser Leu Arg Arg
820 825 830

Arg Ser Val His
835

<210> SEQ_ID NO 4

<211> LENGTH: 1795

<212> TYPE: PRT

<213> ORGANISM: Bifidobacterium bifidum

<220> FEATURE:

<221> NAME/KEY: source

<222> LOCATION: 1..1795

<223> OTHER INFORMATION: /mol_type="protein"
/organism="Bifidobacterium bifidum"

<400> SEQUENCE: 4

Met Thr Thr Ile Phe Arg Arg Ala Thr Ala Lys Thr Leu Met Arg Lys
1 5 10 15

Leu Ser Gly Leu Leu Val Ala Ile Ala Met Leu Ala Val Leu Pro Ala
20 25 30

Gly Thr Ile Ser Ala Asn Ala Ala Asp Glu Pro Pro Gln Glu Tyr Leu
35 40 45

Gln Leu Thr Leu Thr Arg Thr Asp Ser Asn Gly Thr Pro Ala Glu Val
50 55 60

Gly Asp Lys Leu Thr Tyr Ser Leu Gly Tyr Lys Asn Val Ser Asp Thr
65 70 75 80

Gly Phe Ile Val His Pro Thr Ala Ser Asn Leu Asn Asn Val Ala Thr
85 90 95

Pro Gln Ser Ala Ser Asn Pro Asn Pro Met Cys Arg Trp Gly Asn Leu
100 105 110

Ala Ala Gly Ala Ser Ala Ala Cys Thr Trp Ser Ala Ser Lys Glu Phe
115 120 125

Ala Tyr His Val Val Thr Glu Asp Asp Val Ala Asn Gly Phe Thr Pro
130 135 140

Thr Ala Thr Val Ser Ala Thr Thr Gln Asp Gly Thr Asn Gly Val Leu
145 150 155 160

Gln Ser Val Asp Ile Thr Gly Glu Thr Val Pro Ala Val Pro Ala Thr
165 170 175

Ser Thr Leu Arg Val Ala Met Gln Arg Thr Asp Thr Leu Gly Asp Asn
180 185 190

Val Lys Ile Gly Asp Arg Leu Thr Phe Asn Phe Thr Tyr Thr Asn Lys
195 200 205

Thr Ala Gln Lys Ile Tyr Ala Tyr Pro Ser Glu Ser Asn Ile Glu Arg
210 215 220

-continued

Val Asp Val Val Ser Phe Pro Arg Asn Ser Cys Arg Ser Gly Val Glu
225 230 235 240

Ala Asn Gln Thr Ala Ser Cys Gly Phe Ala Tyr His Val Ile Thr Ala
245 250 255

Glu Asp Val Val Ala Arg Arg Tyr Thr Pro Thr Ala Thr Phe Arg Ala
260 265 270

Thr Ser Asp Arg Asp Gly Thr Gln Val Leu Gln Asp Asp Met Thr Phe
275 280 285

Thr Thr Gly Thr Val Thr Val Ala Gly Pro Ala Asp Asp Ala Ala Ser
290 295 300

Thr Pro Thr Glu Arg Lys Asp Gly Glu Pro Leu Leu Leu Ala Thr Asn
305 310 315 320

Lys Gln Ile Gly Asn Thr Asp Tyr Tyr Arg Ile Pro Ala Ile Ala Gln
325 330 335

Ala Pro Asn Gly Trp Ile Leu Ala Ala Trp Asp Leu Arg Pro Lys Leu
340 345 350

Ala Ala Asp Ala Pro Asn Pro Asn Ser Ile Val Gln Arg Ile Ser Lys
355 360 365

Asp Gly Gly Lys Ser Trp Glu Thr Leu Ala Tyr Val Ala Gln Gly Arg
370 375 380

Ser Ala Thr Asn Lys Tyr Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp
385 390 395 400

Glu Glu Ala Gly Lys Ile Phe Leu Phe Cys Val Lys Ser Tyr Asp Gln
405 410 415

Gly Tyr Phe Gly Ser Val Leu Gly Val Glu Asp Ala Arg Asn Val Leu
420 425 430

Gln Ala Val Val Met Glu Ser Asp Asp Asn Gly Ala Thr Trp Ser Glu
435 440 445

Pro Arg Asn Ile Thr Lys Asp Ile Thr Lys Gly His Glu Asp Glu Trp
450 455 460

Lys Ser Arg Phe Ala Ser Ser Gly His Gly Ile Gln Leu Lys Tyr Gly
465 470 475 480

Gln Tyr Lys Gly Arg Leu Ile Gln Gln Tyr Ala Val Arg Thr Thr Ser
485 490 495

Asn Thr Asn Ile Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr
500 505 510

Trp Lys Ala Gly Asn Pro Val Thr Glu Val Asn Met Asp Glu Asn Lys
515 520 525

Val Val Glu Leu Ser Asp Gly Arg Val Met Leu Asn Ser Arg Pro Gly
530 535 540

Ala Ala Gly Tyr Arg Arg Val Ala Ile Ser Glu Asp Gly Val Asn
545 550 555 560

Tyr Gly Pro Ile Lys Ser Glu Thr Gln Leu Pro Asp Pro Asn Asn Asn
565 570 575

Ala Gln Ile Thr Arg Ala Phe Pro Asn Ala Pro Glu Gly Ser Ala Lys
580 585 590

Ala Lys Val Leu Leu Tyr Ser Ala Pro Arg Ala Ser Asn Glu Gly Arg
595 600 605

Ala Asn Gly Val Val Arg Val Ser Phe Asp Asp Gly Thr Thr Trp Ser
610 615 620

Ala Gly Lys Leu Phe Lys Ala Gly Ser Met Ala Tyr Ser Val Ile Thr
625 630 635 640

Ala Leu Asn Asp Ala Ala Gly Gly Tyr Gly Leu Leu Tyr Glu Gly

US 9,102,966 B2

53**54**

-continued

645	650	655
Glu Ser Ile Thr Tyr Thr Arg Ala Ser Met Glu Trp Leu Gly Tyr Leu		
660	665	670
Thr Ala Thr Ala Ser Gly Thr Ala Ala Val Lys Glu Gly Glu Gly Thr		
675	680	685
Leu Thr Ala Pro Val Thr Val Thr Asn Asp Gly Leu Thr Asp Tyr Thr		
690	695	700
Asn Val Thr Val Thr Pro Thr Gly Leu Pro Ser Gly Trp Ser Ala Glu		
705	710	715
Ala Val Asn Val Gly Asn Leu Ala Ala Gly Gln Ser Ala Thr Val Asn		
725	730	735
Val Pro Val Thr Val Pro Ala Ala Ala Val Ser Gly Thr Val Ala Lys		
740	745	750
Ala Thr Met Lys Ile Thr Gly Lys Tyr Ala Gln Ser Glu Asp Thr Leu		
755	760	765
His Ser Phe Ala Glu Gly Glu Leu Ala Val Thr Val Thr Asp Pro Asp		
770	775	780
Pro Ala Ala Lys Arg Leu Lys Leu Thr Ile Glu Arg Thr Asp Asp Asn		
785	790	795
Gly Asp Pro Val Lys Val Gly Asp Thr Leu Thr Tyr Arg Ile Thr Tyr		
805	810	815
Glu Asn Val Gly Thr Gln Ser Phe Ala Val Tyr Pro Arg Glu Ser Asn		
820	825	830
Leu Asp Gly Val Thr Thr Pro Gln Ser Ala Ser Asn Pro Ala Pro Val		
835	840	845
Cys Arg Trp Ser Arg Leu Ala Pro Gly Ala Thr Gly Ala Cys Val Ser		
850	855	860
Gly Asn Gly Lys Gln Leu Ala Tyr His Thr Val Thr Glu Ala Asp Ala		
865	870	875
Thr Ala Gly Ser Phe Thr Pro Ser Ala Thr Ile Asp Ala Thr Ala Asp		
885	890	895
Ala Ser Gly Glu Thr Val Leu Glu Ser Val Ser Ile Thr Gly Asp Pro		
900	905	910
Val Thr Val Ser Gln Pro Val Glu Leu Pro Ala Asp Ile Ala Ala Trp		
915	920	925
Lys Thr Arg Asn Glu Ala Leu Ala Asp Trp Gln Thr Leu Ser Glu Lys		
930	935	940
Leu Ala Lys Thr Asp Arg Ile Asn Trp Leu Phe Thr Gly Asp Ser Ile		
945	950	955
Thr His Gly Val Gln Phe Thr Arg Gly Tyr Arg Thr Tyr Ser Glu Leu		
965	970	975
Phe Ala Asn His Leu Asp Thr Ala Ser Val Arg Gly Val Ser Arg Ala		
980	985	990
Asn Asp Val Val Met Asn Thr Gly Ile Ser Ser Ala Asp Ala Ser Trp		
995	1000	1005
Pro Leu Lys Asp Gly Ala Phe Glu Lys Trp Val Ser Asp Lys His Pro		
1010	1015	1020
Asp Val Val Phe Leu Thr Phe Gly Met Asn Asp Gly Arg Thr Gly Gln		
1025	1030	1035
Ala Phe Thr Val Asp Gln Tyr Thr Ala Asn Leu Ser Thr Leu Ile Asp		
1045	1050	1055
Lys Ile Arg Asp Leu Gly Ala Ile Pro Val Leu Gln Thr Gln Asn Tyr		
1060	1065	1070

-continued

Thr Thr Asn Thr Thr Phe Asn Ala Asn Leu Asp Thr Tyr Phe Asp Ala
 1075 1080 1085
 Glu Arg Arg Leu Ala Leu Asp Lys Asn Val Leu Leu Val Asp Phe Asn
 1090 1095 1100
 Lys Gln Trp Leu Glu Leu Gly Gly Asn Arg Glu Ser Gly Thr Tyr
 1105 1110 1115 1120
 Met Gly Ala Gly Asn Asp Ile His Pro Gly Glu Asn Gly His Ile Glu
 1125 1130 1135
 Trp Ala Lys Phe Thr Leu Gly Ala Leu Asn Met Ile Ala Asn Asp Asp
 1140 1145 1150
 Pro Leu Ala Arg Trp Ser Ser Asp Thr Thr Leu Asp Lys Pro Thr
 1155 1160 1165
 Val Thr Val Asp Ala Asp Gly Asn Gly Leu Lys Gly Ser Asp Gly Leu
 1170 1175 1180
 Glu Pro Ala Pro Ala Ala Lys Ser Val Gly Lys Phe Leu Ser Gly
 1185 1190 1195 1200
 Ala Gln Tyr Val Asp Leu Gly Gly Asp Val Val Ser Ala Val Ala Gly
 1205 1210 1215
 Lys Arg Glu Ser Asn Val Thr Ile Arg Phe Arg Ala Ser Ala Thr Gly
 1220 1225 1230
 Gln Pro Gln Thr Leu Phe Ser Leu Gly Asp Ser Asp Ser Ala Thr Arg
 1235 1240 1245
 Ala Thr Val Arg Leu Ser Ala Thr Gly Leu Val Gln Phe Leu Asn Ser
 1250 1255 1260
 Gly Asn Thr Gly Asp Phe Tyr Thr Val Gly Thr Asn Asp Leu Ala Asp
 1265 1270 1275 1280
 Gly Ala Trp His Thr Val Ser Val Asn Phe Val Ala Asn Gly Phe Thr
 1285 1290 1295
 Ile Tyr Val Asp Gly Ala Ala Met Arg Ala Ile Ser Gly Gly Ala Gly
 1300 1305 1310
 Thr Gln Leu Asn Val Pro Gly Ala Ile Thr Val Asn Thr Ala Thr Ala
 1315 1320 1325
 Gly Ala Ile Arg Gly Ala Asp Ser Ala Gly Gly Ala Gln Gln Leu Thr
 1330 1335 1340
 Gly Ile Val Asp Tyr Val Ala Ala Trp Ser Arg Thr Leu Thr Asp Ala
 1345 1350 1355 1360
 Glu Ala Lys Arg Ile Ser Ala Glu Thr Ser Ala Val Ala Val Thr Lys
 1365 1370 1375
 Val Asp Ala Ala Val Asn Ala Leu Gln Pro Ile Ile Ser Asp Thr Gly
 1380 1385 1390
 Ala Arg Lys Asn Ile Val Phe Val Gly Gly Glu Thr Ile Glu Gly Gly
 1395 1400 1405
 Tyr Thr Asp His Leu Ile Ala Lys Asn Ile Val Gln Leu Leu Asp Glu
 1410 1415 1420
 Arg Val Arg Trp Glu Tyr Val Thr Gly Leu Ser Ala Thr Asp Arg Glu
 1425 1430 1435 1440
 Arg Gln Arg Ala Lys Phe Phe Val Ala Ala Gly Gln Gly Leu Thr
 1445 1450 1455
 Ala Lys Gln Met Asp Glu Asp Tyr Ala Ala Met Val Gly Glu Tyr Ser
 1460 1465 1470
 Pro Asp Ile Leu Phe Leu Ala Pro Asp Leu Tyr Asp Ala Asp Gly Asn
 1475 1480 1485

-continued

Leu Ala Glu Ser Ala Ala Ala Ala Phe Ala Gly His Ile Arg Ser Val
 1490 1495 1500
 Ala Ala Lys Ala Lys Glu Ala Gly Ala Lys Val Val Leu Val Thr Pro
 1505 1510 1515 1520
 Val Thr Val Arg Gly Gly Glu Asp Glu Tyr Ala Gly Ala Met Arg Thr
 1525 1530 1535
 Val Ala Lys Glu Asp Asp Leu Pro Leu Ile Asp Ala Gln Ala Trp Ile
 1540 1545 1550
 Gly Lys Val Val Ala Ala Asp Ala Ser Val Lys Thr Ala Trp Phe Asn
 1555 1560 1565
 Lys Ala Gly Gln Leu Asn Tyr Ala Gly His Leu Gly Tyr Ala Arg Phe
 1570 1575 1580
 Met Met Arg Ser Leu Asp Leu Tyr Pro Ser Asn Val Ser Gly Ser Arg
 1585 1590 1595 1600
 Ile Ala Ser Leu Pro Tyr Asp Thr Ala Asn Val Thr Leu Val Gly Ala
 1605 1610 1615
 Ser Glu Asn Gly Glu Leu Pro Val Gly Arg Val Glu Gly Thr Asp
 1620 1625 1630
 Arg Ala His Ile Asp Thr Met Gln Ile Gly Ala Ala Ala Ser Leu Val
 1635 1640 1645
 Val Val Asp Ser Tyr Ala Val Tyr Glu Ile Gly Glu Asp Gly Gly Arg
 1650 1655 1660
 Thr Leu Val Ala Asp Gly Leu Lys Pro Ala Asp Val Leu Ala Asp Gly
 1665 1670 1675 1680
 Ile Asp Val Thr Val Asn Asp Thr Ala Ala His Arg Tyr Glu Val Val
 1685 1690 1695
 Gly Ser Ala Asn Val Pro Glu Gly Ala Asp Ala Val Thr Val Thr Tyr
 1700 1705 1710
 Thr Ala Thr Leu Ala Ala Val Glu Glu Pro Glu Pro Gly Pro Asp Pro
 1715 1720 1725
 Asp Pro Thr Pro Asp Pro Ser Glu Lys Pro Asp Gly Asp Gly Thr Gly
 1730 1735 1740
 Asp Gly Thr Gly Ala Gly Thr Gly Asp Val Gln Lys Pro Thr Pro Asp
 1745 1750 1755 1760
 Ala Val Ala Lys Thr Gly Ala Asp Val Phe Gly Leu Leu Thr Ala Val
 1765 1770 1775
 Ala Ala Leu Leu Ala Ala Gly Gly Val Thr Leu Ser Leu Arg Arg Arg
 1780 1785 1790
 Ala Asn Arg
 1795

```

<210> SEQ_ID NO 5
<211> LENGTH: 1795
<212> TYPE: PRT
<213> ORGANISM: Bifidobacterium bifidum
<220> FEATURE:
<221> NAME/KEY: source
<222> LOCATION: 1..1795
<223> OTHER INFORMATION: /mol_type="protein"
/organism="Bifidobacterium bifidum"

<400> SEQUENCE: 5

Met Thr Thr Ile Phe Arg Arg Ala Thr Ala Lys Thr Leu Met Arg Lys
1 5 10 15

Leu Ser Gly Leu Leu Val Ala Ile Ala Met Leu Ala Val Leu Pro Ala
20 25 30
  
```

US 9,102,966 B2

59**60**

-continued

Gly Thr Ile Ser Ala Asn Ala Ala Asp Glu Pro Pro Gln Glu Tyr Leu
35 40 45

Gln Leu Thr Leu Thr Arg Thr Asp Ser Asn Gly Thr Pro Ala Glu Val
50 55 60

Gly Asp Lys Leu Thr Tyr Ser Leu Gly Tyr Lys Asn Val Ser Asp Thr
65 70 75 80

Gly Phe Ile Val His Pro Thr Ala Ser Asn Leu Asn Asn Val Ala Thr
85 90 95

Pro Gln Ser Ala Ser Asn Pro Asn Pro Met Cys Arg Trp Gly Asn Leu
100 105 110

Ala Ala Gly Ala Ser Ala Ala Cys Thr Trp Ser Ala Ser Lys Glu Phe
115 120 125

Ala Tyr His Val Val Thr Glu Asp Asp Val Ala Asn Gly Phe Thr Pro
130 135 140

Thr Ala Thr Val Ser Ala Thr Thr Gln Asp Gly Thr Asn Gly Val Leu
145 150 155 160

Gln Ser Val Asp Ile Thr Gly Glu Thr Val Pro Ala Val Pro Ala Thr
165 170 175

Ser Thr Leu Arg Val Ala Met Gln Arg Thr Asp Thr Leu Gly Asp Asn
180 185 190

Val Lys Ile Gly Asp Arg Leu Thr Phe Asn Phe Thr Tyr Thr Asn Lys
195 200 205

Thr Ala Gln Lys Ile Tyr Ala Tyr Pro Ser Glu Ser Asn Ile Glu Arg
210 215 220

Val Asp Val Val Ser Phe Pro Arg Asn Ser Cys Arg Ser Gly Val Glu
225 230 235 240

Ala Asn Gln Thr Ala Ser Cys Gly Phe Ala Tyr His Val Ile Thr Ala
245 250 255

Glu Asp Val Ala Arg Arg Tyr Thr Pro Thr Ala Thr Phe Arg Ala
260 265 270

Thr Ser Asp Arg Asp Gly Thr Gln Val Leu Gln Asp Asp Met Thr Phe
275 280 285

Thr Thr Gly Thr Val Thr Val Ala Gly Pro Ala Asp Asp Ala Ala Ser
290 295 300

Thr Pro Thr Glu Arg Lys Asp Gly Glu Pro Leu Leu Leu Ala Thr Asn
305 310 315 320

Lys Gln Ile Gly Asn Thr Asp Tyr Tyr Arg Ile Pro Ala Ile Ala Gln
325 330 335

Ala Pro Asn Gly Trp Ile Leu Ala Ala Trp Asp Leu Arg Pro Lys Leu
340 345 350

Ala Ala Asp Ala Pro Asn Pro Asn Ser Ile Val Gln Arg Ile Ser Lys
355 360 365

Asp Gly Gly Lys Ser Trp Glu Thr Leu Ala Tyr Val Ala Gln Gly Arg
370 375 380

Ser Ala Thr Asn Lys Tyr Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp
385 390 395 400

Glu Glu Ala Gly Lys Ile Phe Leu Phe Cys Val Lys Ser Tyr Asp Gln
405 410 415

Gly Tyr Phe Gly Ser Val Leu Gly Val Glu Asp Ala Arg Asn Val Leu
420 425 430

Gln Ala Val Val Met Glu Ser Asp Asp Asn Gly Ala Thr Trp Ser Glu
435 440 445

Pro Arg Asn Ile Thr Lys Asp Ile Thr Lys Gly His Glu Asp Glu Trp

US 9,102,966 B2

61**62**

-continued

450	455	460
Lys Ser Arg Phe Ala Ser Ser Gly His Gly Ile Gln Leu Lys Tyr Gly		
465	470	475
Gln Tyr Lys Gly Arg Leu Ile Gln Gln Tyr Ala Val Arg Thr Thr Ser		
485	490	495
Asn Thr Asn Ile Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr		
500	505	510
Trp Lys Ala Gly Asn Pro Val Thr Glu Ala Asn Met Asp Glu Asn Lys		
515	520	525
Val Val Glu Leu Ser Asp Gly Arg Val Met Leu Asn Ser Arg Pro Gly		
530	535	540
Ala Ala Gly Tyr Arg Arg Val Ala Ile Ser Glu Asp Gly Gly Val Asn		
545	550	555
Tyr Gly Pro Ile Lys Ser Glu Thr Gln Leu Pro Asp Pro Asn Asn Asn		
565	570	575
Ala Gln Ile Thr Arg Ala Phe Pro Asn Ala Pro Glu Gly Ser Ala Lys		
580	585	590
Ala Lys Val Leu Leu Tyr Ser Ala Pro Arg Ala Ser Asn Glu Gly Arg		
595	600	605
Ala Asn Gly Val Val Arg Val Ser Phe Asp Asp Gly Thr Thr Trp Ser		
610	615	620
Ala Gly Lys Leu Phe Lys Glu Gly Ser Met Ala Tyr Ser Val Ile Thr		
625	630	635
640		
Ala Leu Asn Asp Ala Ala Gly Gly Tyr Gly Leu Leu Tyr Glu Gly		
645	650	655
Glu Ser Ile Thr Tyr Thr Arg Val Ser Met Glu Trp Leu Gly Tyr Leu		
660	665	670
Thr Ala Thr Ala Ser Gly Thr Ala Thr Val Lys Glu Gly Glu Gly Thr		
675	680	685
Leu Thr Ala Pro Val Thr Val Thr Asn Asp Gly Leu Thr Asp Tyr Thr		
690	695	700
Asn Val Thr Val Thr Pro Thr Gly Leu Pro Ser Gly Trp Ser Ala Glu		
705	710	715
720		
Ala Val Asn Val Gly Asn Leu Ala Ala Gly Gln Ser Ala Thr Val Asn		
725	730	735
Val Pro Val Thr Val Pro Ala Ala Ala Val Ser Gly Thr Val Ala Lys		
740	745	750
Ala Thr Met Lys Ile Thr Gly Lys Tyr Ala Gln Ser Glu Asp Thr Leu		
755	760	765
His Ser Phe Ala Glu Gly Glu Leu Ala Val Thr Val Thr Glu Pro Asp		
770	775	780
Pro Ala Ala Lys Arg Leu Lys Leu Thr Ile Glu Arg Thr Asp Asp Asn		
785	790	795
800		
Gly Ala Pro Val Lys Val Gly Asp Thr Leu Thr Tyr Arg Ile Thr Tyr		
805	810	815
Glu Asn Val Gly Thr Gln Ser Phe Ala Val Tyr Pro Arg Lys Ser Asn		
820	825	830
Leu Asp Gly Val Thr Thr Pro Gln Ser Ala Ser Asn Pro Ala Pro Val		
835	840	845
Cys Arg Trp Ser Arg Leu Asp Pro Gly Thr Thr Gly Ala Cys Val Ser		
850	855	860
Gly Asn Gly Lys Arg Leu Ala Tyr His Thr Val Thr Glu Ala Asp Ala		
865	870	875
880		

-continued

Thr Ala Gly Ser Phe Thr Pro Ser Ala Thr Ile Asp Ala Thr Ala Asp
 885 890 895
 Thr Ser Gly Glu Thr Val Leu Glu Ser Val Ser Ile Thr Gly Asp Pro
 900 905 910
 Val Thr Val Ser Gln Pro Val Glu Leu Pro Ala Asp Ile Ala Ala Trp
 915 920 925
 Lys Thr Arg Asn Glu Ala Leu Asp Asp Trp Gln Thr Leu Ser Glu Lys
 930 935 940
 Leu Ala Lys Thr Asp Arg Ile Asn Trp Leu Phe Thr Gly Asp Ser Ile
 945 950 955 960
 Thr His Gly Val Gln Leu Thr Arg Gly Tyr Arg Thr Tyr Ser Glu Leu
 965 970 975
 Phe Ala Asn His Leu Asp Thr Ala Ser Val Arg Gly Val Ser Arg Ala
 980 985 990
 Asn Asp Val Val Met Asn Thr Gly Ile Ser Ser Ala Asp Ala Ser Trp
 995 1000 1005
 Pro Leu Lys Asp Gly Ala Phe Glu Lys Trp Val Ser Asp Lys His Pro
 1010 1015 1020
 Asp Val Val Phe Leu Thr Phe Gly Met Asn Asp Gly Arg Thr Gly Gln
 1025 1030 1035 1040
 Ala Phe Thr Val Asp Gln Tyr Thr Ala Asn Leu Ser Thr Leu Ile Asp
 1045 1050 1055
 Lys Ile Arg Asp Leu Gly Ala Ile Pro Val Leu Gln Thr Gln Asn Tyr
 1060 1065 1070
 Thr Thr Asn Thr Thr Phe Asn Ala Asn Leu Asp Thr Tyr Phe Asp Ala
 1075 1080 1085
 Glu Arg Arg Leu Ala Leu Asp Lys Asn Val Leu Leu Val Asp Phe Asn
 1090 1095 1100
 Lys Gln Trp Leu Glu Leu Gly Gly Asn Arg Glu Ser Gly Thr Tyr
 1105 1110 1115 1120
 Met Gly Ala Gly Asn Asp Ile His Pro Gly Glu Asn Gly His Ile Glu
 1125 1130 1135
 Trp Ala Lys Phe Thr Leu Gly Ala Leu Asn Met Ile Ala Asn Asp Asp
 1140 1145 1150
 Pro Leu Ala Arg Trp Ser Ser Asp Thr Thr Leu Asp Lys Pro Thr
 1155 1160 1165
 Val Thr Val Asp Ala Asp Gly Asn Gly Leu Lys Gly Ser Asp Gly Leu
 1170 1175 1180
 Glu Pro Ala Pro Ala Ala Lys Ser Val Gly Lys Phe Leu Ser Gly
 1185 1190 1195 1200
 Ala Gln Tyr Val Asp Leu Gly Gly Asp Val Val Ser Ala Val Ala Gly
 1205 1210 1215
 Lys Arg Glu Ser Asn Val Thr Ile Arg Phe Arg Ala Ser Ala Thr Gly
 1220 1225 1230
 Gln Pro Gln Thr Leu Phe Ser Leu Gly Asp Ser Asp Ser Ala Thr Arg
 1235 1240 1245
 Ala Thr Val Arg Leu Ser Ala Thr Gly Leu Val Gln Phe Leu Asn Ser
 1250 1255 1260
 Gly Asn Thr Gly Asp Phe Tyr Thr Val Gly Thr Asn Asp Leu Ala Asp
 1265 1270 1275 1280
 Gly Ala Trp His Thr Val Ser Val Asn Phe Val Ala Asn Gly Phe Thr
 1285 1290 1295

-continued

Ile Tyr Val Asp Gly Ala Ala Met Arg Ala Ile Ser Gly Gly Ala Gly
1300 1305 1310

Thr Gln Leu Asn Val Pro Gly Ala Ile Thr Val Asn Thr Ala Thr Ala
1315 1320 1325

Gly Ala Ile Arg Gly Ala Asp Ser Ala Gly Gly Ala Gln Gln Leu Thr
1330 1335 1340

Gly Ile Val Asp Tyr Val Ala Ala Trp Ser Arg Thr Leu Thr Asp Ala
1345 1350 1355 1360

Glu Ala Lys Arg Ile Ser Ala Glu Thr Ser Ala Val Ala Val Thr Lys
1365 1370 1375

Val Asp Ala Ala Val Asn Ala Leu Gln Pro Ile Ile Ser Asp Thr Gly
1380 1385 1390

Ala Arg Lys Asn Ile Val Phe Val Gly Gly Glu Thr Ile Glu Gly Gly
1395 1400 1405

Tyr Thr Asp His Leu Ile Ala Lys Asn Ile Val Gln Leu Leu Asp Glu
1410 1415 1420

Arg Val Arg Trp Glu Tyr Val Thr Gly Leu Ser Ala Thr Asp Arg Glu
1425 1430 1435 1440

Leu Gln Arg Ala Lys Phe Phe Val Ala Ala Gly Gln Gly Gly Leu Thr
1445 1450 1455

Ala Lys Gln Met Asp Glu Asp Tyr Ala Ala Met Val Gly Glu Tyr Ser
1460 1465 1470

Pro Asp Ile Leu Phe Leu Ala Pro Asp Leu Tyr Asp Ala Asp Gly Ile
1475 1480 1485

Leu Ala Glu Ser Asp Ala Ala Ala Phe Ala Gly His Ile Arg Ser Val
1490 1495 1500

Ala Ala Lys Ala Lys Glu Ala Ala Gly Ala Lys Val Val Leu Val Thr Pro
1505 1510 1515 1520

Val Thr Val Arg Gly Glu Asp Glu Tyr Ala Gly Ala Met Arg Thr
1525 1530 1535

Val Ala Lys Glu Asp Asp Leu Pro Leu Ile Asp Ala Gln Ala Trp Ile
1540 1545 1550

Gly Lys Val Val Ala Ala Asp Ala Ser Val Lys Thr Ala Trp Phe Asn
1555 1560 1565

Lys Ala Gly Gln Leu Asn Tyr Ala Gly His Leu Gly Tyr Ala Arg Phe
1570 1575 1580

Met Met Arg Ser Leu Asp Leu Tyr Pro Ser Asn Val Ser Gly Ser Arg
1585 1590 1595 1600

Ile Ala Ser Leu Pro Tyr Asp Thr Ala Asn Val Thr Leu Val Gly Ala
1605 1610 1615

Ser Glu Asn Gly Glu Leu Pro Val Gly Arg Val Glu Gly Thr Asp
1620 1625 1630

Arg Ala His Ile Asp Thr Met Gln Ile Gly Ala Ala Ala Ser Leu Val
1635 1640 1645

Val Thr Asp Ser Tyr Ala Val Tyr Glu Ile Gly Glu Asp Gly Gly Arg
1650 1655 1660

Thr Leu Val Ala Asp Gly Leu Lys Pro Ala Asp Val Leu Ala Asp Gly
1665 1670 1675 1680

Ile Asp Val Thr Val Asn Asp Thr Ala Ala His Arg Tyr Glu Val Val
1685 1690 1695

Gly Ser Ala Asn Val Pro Glu Gly Ala Asp Ala Val Thr Val Thr Tyr
1700 1705 1710

Thr Ala Thr Leu Ala Ala Val Glu Glu Pro Glu Pro Gly Pro Asp Pro

-continued

1715	1720	1725
Asp Pro Thr Pro Asp Pro Ser Glu Lys Pro Asp Gly Asp Gly Thr Gly		
1730	1735	1740
Asp Gly Thr Gly Ala Gly Ala Gly Asp Val Gln Lys Pro Thr Pro Asp		
1745	1750	1755
Ala Val Ala Lys Thr Gly Ala Asp Val Phe Gly Leu Leu Ala Ala Val		
1765	1770	1775
Ala Val Leu Leu Ala Ala Gly Gly Val Thr Leu Ser Leu Arg Arg Arg		
1780	1785	1790
Ala Asn Arg		
1795		

<210> SEQ ID NO 6
<211> LENGTH: 770
<212> TYPE: PRT
<213> ORGANISM: *Bifidobacterium bifidum*
<220> FEATURE:
<221> NAME/KEY: source
<222> LOCATION: 1..770
<223> OTHER INFORMATION: /mol_type="protein"
/organism="*Bifidobacterium bifidum*"
<400> SEQUENCE: 6

Met Val Arg Ser Thr Lys Pro Ser Leu Leu Arg Arg Phe Gly Ala Leu		
1	5	10
Val Ala Ala Ala Ala Met Leu Val Val Leu Pro Ala Gly Val Ser Thr		
20	25	30
Ala Ser Ala Ala Ser Asp Asp Ala Asp Met Leu Thr Val Thr Met Thr		
35	40	45
Arg Thr Asp Ala Leu Gly Asp Glu Val Tyr Val Gly Asp Thr Leu Thr		
50	55	60
Tyr Ser Phe Thr Asn Thr Asn Asn Thr Ser Ser Ala Phe Thr Ala Phe		
65	70	75
Pro Ala Glu Ser Asn Leu Ser Gly Val Leu Thr Thr Gly Thr Pro Asn		
85	90	95
Cys Arg Tyr Glu Asn Leu Ala Gly Gly Ala Ser Tyr Pro Cys Ser Thr		
100	105	110
Ala Ser His Thr Ile Thr Ala Asp Asp Leu Thr Ala Gly Ser Phe Thr		
115	120	125
Pro Arg Thr Val Trp Lys Ala Thr Ser Asp Arg Gly Gly Thr Gln Val		
130	135	140
Leu Gln Asp Asn Ile Val Ser Thr Gly Asp Thr Val Thr Val Lys Glu		
145	150	155
Gly Lys Arg Pro Asp Pro Ala Thr Ile Pro Thr Asp Arg Ala Asp Gly		
165	170	175
Glu Ala Val Arg Leu Ala Thr Ala Arg Gln Asn Leu Gly Thr Glu Cys		
180	185	190
Tyr Arg Ile Pro Ala Leu Ala Glu Ala Pro Asn Gly Trp Ile Leu Ala		
195	200	205
Ala Phe Asp Gln Arg Pro Asn Thr Ala Met Ala Asn Gly Ser Gly Val		
210	215	220
Lys Cys Trp Asp Ala Pro Gln Pro Asn Ser Ile Val Gln Arg Ile Ser		
225	230	235
Lys Asp Gly Gly Lys Ser Trp Thr Pro Ile Gln Tyr Val Ala Gln Gly		
245	250	255
Lys Asn Ala Pro Glu Arg Tyr Gly Tyr Ser Asp Pro Ser Tyr Val Val		

-continued

260	265	270
Asp Lys Glu Thr Gly Glu Ile Phe Leu Phe Phe Val His Ser Tyr Asn		
275	280	285
Lys Gly Phe Ala Asp Ser Gln Leu Gly Val Asp Glu Ser Asn Arg Asn		
290	295	300
Val Leu His Ala Val Val Val Ser Ser Lys Asp Asn Gly Glu Thr Trp		
305	310	315
Ser Lys Pro Arg Asp Ile Thr Ala Asp Ile Thr Lys Gly Tyr Glu Asn		
325	330	335
Glu Trp Lys Ser Arg Phe Ala Thr Ser Gly Ala Gly Ile Gln Leu Lys		
340	345	350
Tyr Gly Lys Tyr Lys Gly Arg Leu Ile Gln Gln Tyr Ala Val Gly Arg		
355	360	365
Thr Thr Gly Ser Asn Ala Ala Val Ser Val Tyr Ser Asp Asp His Gly		
370	375	380
Lys Thr Trp Gln Ala Gly Asn Pro Val Thr Gly Met Leu Met Asp Glu		
385	390	395
Asn Lys Val Val Glu Leu Ser Asp Gly Arg Val Met Leu Asn Ser Arg		
405	410	415
Pro Gly Asn Gly Ser Gly Tyr Arg Arg Val Ala Ile Ser Glu Asp Gly		
420	425	430
Gly Val Asn Tyr Gly Thr Val Lys Asn Glu Thr Gln Leu Pro Asp Pro		
435	440	445
Asn Asn Asn Ala His Ile Thr Arg Ala Phe Pro Asn Ala Pro Glu Gly		
450	455	460
Ser Ala Lys Ala Lys Val Leu Leu Tyr Ser Ser Pro Arg Ala Asn Asn		
465	470	475
Glu Gly Arg Ala Asn Gly Val Val Arg Ile Ser Leu Asp Asp Gly Thr		
485	490	495
Thr Trp Ser Ser Gly Lys Leu Tyr Lys Glu Gly Ser Met Ala Tyr Ser		
500	505	510
Val Ile Thr Ala Leu Ser Gly Ala Ala Gly Gly Tyr Gly Leu Leu		
515	520	525
Tyr Glu Gly Ala Trp Val Thr Gly Gly Ile Asp Ser His Asp Ile		
530	535	540
Met Tyr Thr His Ile Ser Met Asp Trp Leu Gly Tyr Leu Ser Ala Thr		
545	550	555
Ala Asp Asp Val Thr Ala Ser Val Glu Glu Gly Ala Ser Thr Val Asp		
565	570	575
Val Thr Val Pro Val Ser Asn Val Gly Ser Val Asp Tyr Thr Gly Val		
580	585	590
Thr Val Thr Pro Ala Asp Leu Pro Thr Gly Trp Ser Ala Ser Pro Val		
595	600	605
Asn Val Gly Ala Leu Ala Ser Gly Ala Ser Lys Asp Val Thr Val Thr		
610	615	620
Val Asn Val Pro Ala Thr Ala Lys Lys Asp Asp Val Ala Lys Ile Val		
625	630	635
Leu Arg Val Thr Gly Thr Ser Ala Ala Asn Ala Asn Ala Thr Thr Gly		
645	650	655
Phe Asp Gly Ser Ile Thr Val Asn Val Thr Glu Lys Ser Glu Pro Asp		
660	665	670
Pro Glu Pro Glu Pro Thr Ile Thr Gly Val Ser Ala Val Thr Ser Gln		
675	680	685

-continued

Ala Gly Val Lys Val Gly Asp Val Phe Asp Ala Ser Lys Val Ser Val
690 695 700

Thr Ala Ala Met Ser Asp Gly Ser Ser Lys Ala Leu Ala Ala Gly Glu
705 710 715 720

Tyr Ser Leu Ser Ala Val Asp Ala Asp Gly Lys Ala Val Asp Leu Ala
725 730 735

Glu Pro Phe Ala Ala Ala Gly Val Val Thr Val Thr Val Ser Val Pro
740 745 750

Val Glu Gly Ala Asp Pro Leu Thr Ala Ser Phe Thr Ile Asp Val Ala
755 760 765

Glu Lys
770

<210> SEQ ID NO 7

<211> LENGTH: 834

<212> TYPE: PRT

<213> ORGANISM: Bifidobacterium bifidum

<220> FEATURE:

<221> NAME/KEY: source

<222> LOCATION: 1..834

<223> OTHER INFORMATION: /mol_type="protein"
/organism="Bifidobacterium bifidum"

<400> SEQUENCE: 7

Met Val Arg Ser Thr Lys Pro Ser Leu Leu Arg Arg Leu Gly Ala Leu
1 5 10 15

Val Ala Ala Ala Ala Met Leu Val Val Leu Pro Ala Gly Val Ser Thr
20 25 30

Ala Ser Ala Ala Ser Asp Asp Ala Asp Met Leu Thr Val Thr Met Thr
35 40 45

Arg Thr Asp Ala Leu Gly Asp Glu Val Tyr Val Gly Asp Thr Leu Thr
50 55 60

Tyr Ser Phe Thr Asn Thr Asn Asn Thr Ser Ser Ala Phe Thr Ala Phe
65 70 75 80

Pro Ala Glu Ser Asn Leu Ser Gly Val Leu Thr Thr Gly Thr Pro Asn
85 90 95

Cys Arg Tyr Glu Asn Leu Ala Gly Gly Ala Ser Tyr Pro Cys Ser Thr
100 105 110

Ala Ser His Thr Ile Thr Ala Asp Asp Leu Thr Ala Gly Ser Phe Thr
115 120 125

Pro Arg Thr Val Trp Lys Ala Thr Ser Asp Arg Gly Gly Thr Gln Val
130 135 140

Leu Gln Asp Asn Ile Val Ser Thr Gly Asp Thr Val Thr Val Lys Glu
145 150 155 160

Gly Lys Arg Pro Asp Pro Ala Thr Ile Pro Thr Asp Arg Ala Asp Gly
165 170 175

Glu Ala Val Arg Leu Ala Thr Ala Arg Gln Asn Leu Gly Thr Glu Cys
180 185 190

Tyr Arg Ile Pro Ala Leu Ala Glu Ala Pro Asn Gly Trp Ile Leu Ala
195 200 205

Ala Phe Asp Gln Arg Pro Asn Thr Ala Met Ala Asn Gly Ser Gly Val
210 215 220

Lys Cys Trp Asp Ala Pro Gln Pro Asn Ser Ile Val Gln Arg Ile Ser
225 230 235 240

Lys Asp Gly Gly Lys Ser Trp Thr Pro Ile Gln Tyr Val Ala Gln Gly
245 250 255

-continued

Lys Asn Ala Pro Glu Arg Tyr Gly Tyr Ser Asp Pro Ser Tyr Val Val
260 265 270

Asp Glu Glu Thr Gly Glu Ile Phe Leu Phe Phe Val His Ser Tyr Asn
275 280 285

Lys Gly Phe Ala Asp Ser Gln Leu Gly Val Asp Glu Ser Asn Arg Asn
290 295 300

Val Leu His Ala Val Val Val Ser Ser Lys Asp Asn Gly Glu Thr Trp
305 310 315 320

Ser Lys Pro Arg Asp Ile Thr Ala Asp Ile Thr Lys Gly Tyr Glu Asn
325 330 335

Glu Trp Lys Ser Arg Phe Ala Thr Ser Gly Ala Gly Ile Gln Leu Lys
340 345 350

Tyr Gly Lys Tyr Lys Gly Arg Leu Ile Gln Gln Tyr Ala Val Gly Arg
355 360 365

Thr Thr Gly Ser Asn Ala Ala Val Ser Val Tyr Ser Asp Asp His Gly
370 375 380

Lys Thr Trp Gln Ala Gly Asn Pro Val Thr Gly Met Leu Met Asp Glu
385 390 395 400

Asn Lys Val Val Glu Leu Ser Asp Gly Arg Val Met Leu Asn Ser Arg
405 410 415

Pro Gly Asn Gly Ser Gly Tyr Arg Arg Val Ala Ile Ser Lys Asp Gly
420 425 430

Gly Val Asn Tyr Gly Thr Val Lys Asn Glu Thr Gln Leu Pro Asp Pro
435 440 445

Asn Asn Asn Ala His Ile Thr Arg Ala Phe Pro Asn Ala Pro Glu Gly
450 455 460

Ser Ala Lys Ala Lys Val Leu Leu Tyr Ser Ser Pro Arg Ala Asn Asn
465 470 475 480

Glu Gly Arg Ala Asn Gly Val Val Arg Ile Ser Leu Asp Asp Gly Thr
485 490 495

Thr Trp Ser Ser Gly Lys Leu Tyr Lys Ala Gly Ser Met Ala Tyr Ser
500 505 510

Val Ile Thr Ala Leu Ser Gly Ala Ala Gly Gly Tyr Gly Leu Leu
515 520 525

Tyr Glu Gly Ala Trp Val Thr Gly Gly Ile Asp Ser His Asp Ile
530 535 540

Met Tyr Thr His Ile Ser Met Asp Trp Leu Gly Tyr Leu Ser Ala Thr
545 550 555 560

Ala Asp Asp Val Thr Ala Ser Val Glu Glu Ala Ser Thr Val Asp
565 570 575

Val Thr Val Pro Val Ser Asn Ile Gly Ser Val Asp Tyr Thr Gly Val
580 585 590

Thr Val Thr Pro Ala Asp Leu Pro Thr Gly Trp Ser Ala Ser Pro Val
595 600 605

Asn Val Gly Ala Leu Ala Ser Gly Ala Ser Lys Asp Val Thr Val Thr
610 615 620

Val Asn Val Pro Ala Thr Ala Lys Lys Asp Asp Val Ala Lys Ile Val
625 630 635 640

Leu Arg Val Thr Gly Thr Ser Ala Ala Asn Ala Asp Ala Thr Thr Gly
645 650 655

Phe Asp Gly Ser Ile Thr Val Asn Val Thr Glu Lys Ser Glu Pro Asp
660 665 670

-continued

Pro Glu Pro Glu Pro Thr Ile Thr Gly Val Ser Ala Val Thr Ser Gln
 675 680 685

Ala Gly Val Lys Val Gly Asp Val Phe Asp Ala Ser Lys Val Ser Val
 690 695 700

Thr Ala Ala Met Ser Asp Gly Ser Ser Lys Ala Leu Ala Ala Gly Glu
 705 710 715 720

Tyr Ser Leu Ser Ala Val Asp Ala Asp Gly Lys Ala Val Asp Leu Ala
 725 730 735

Glu Pro Phe Ala Ala Ala Gly Val Val Thr Val Thr Val Ser Val Pro
 740 745 750

Val Glu Gly Ala Asn Pro Leu Thr Ala Ser Phe Thr Ile Asp Val Ala
 755 760 765

Glu Lys Ser Val Asp Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro
 770 775 780

Glu Lys Pro Ala Gly Pro Lys Val Asp Val Pro Thr Glu Gln Pro Gly
 785 790 795 800

Leu Ser Lys Thr Gly Ala Ser Thr Ala Gly Met Ser Ile Val Phe Val
 805 810 815

Leu Leu Ala Leu Ser Gly Val Ala Ala Leu Ser Leu Arg Arg Arg Ser
 820 825 830

Ala His

<210> SEQ ID NO 8

<211> LENGTH: 1782

<212> TYPE: PRT

<213> ORGANISM: Bifidobacterium bifidum

<220> FEATURE:

<221> NAME/KEY: source

<222> LOCATION: 1..1782

<223> OTHER INFORMATION: /mol_type="protein"
 /organism="Bifidobacterium bifidum"

<400> SEQUENCE: 8

Met Arg Lys Leu Ser Gly Leu Leu Val Ala Ile Ala Met Leu Ala Val
 1 5 10 15

Leu Pro Ala Gly Thr Ile Ser Ala Asn Ala Ala Asp Glu Pro Pro Gln
 20 25 30

Glu Tyr Leu Gln Leu Thr Leu Thr Arg Thr Asp Ser Asn Gly Thr Pro
 35 40 45

Ala Glu Val Gly Asp Lys Leu Thr Tyr Ser Leu Gly Tyr Lys Asn Val
 50 55 60

Ser Asp Thr Gly Phe Ile Val His Pro Thr Ala Ser Asn Leu Asn Asn
 65 70 75 80

Val Ala Thr Pro Gln Ser Ala Ser Asn Pro Asn Pro Met Cys Arg Trp
 85 90 95

Gly Asn Leu Ala Ala Gly Ala Ser Ala Ala Cys Thr Trp Ser Ala Ser
 100 105 110

Lys Glu Phe Ala Tyr His Val Val Thr Glu Asp Asp Val Ala Asn Gly
 115 120 125

Phe Thr Pro Thr Ala Thr Val Ser Ala Thr Thr Gln Asp Gly Thr Asn
 130 135 140

Gly Val Leu Gln Ser Val Asp Ile Thr Gly Glu Thr Val Pro Ala Val
 145 150 155 160

Pro Ala Thr Ser Thr Leu Arg Val Ala Met Gln Arg Thr Asp Thr Leu
 165 170 175

Gly Asp Asn Val Lys Ile Gly Asp Arg Leu Thr Phe Asn Phe Thr Tyr

US 9,102,966 B2

77

-continued

78

180	185	190
Thr Asn Lys Thr Ala Gln Lys Ile Tyr Ala Tyr Pro Ser Glu Ser Asn		
195	200	205
Ile Glu Arg Val Asp Val Val Ser Tyr Pro Arg Asn Ser Cys Arg Ser		
210	215	220
Gly Val Glu Ala Asn Gln Thr Ala Ser Cys Gly Phe Ala Tyr His Val		
225	230	235
Ile Thr Ala Glu Asp Val Val Ala Arg Arg Tyr Thr Pro Thr Ala Thr		
245	250	255
Phe Arg Ala Thr Ser Asp Arg Asp Gly Thr Gln Val Leu Gln Asp Asp		
260	265	270
Met Thr Phe Thr Thr Gly Thr Val Thr Val Ala Gly Pro Ala Asp Asp		
275	280	285
Ala Ala Ser Thr Pro Thr Glu Arg Lys Asp Gly Glu Pro Leu Leu Leu		
290	295	300
Ala Thr Asn Lys Gln Ile Gly Asn Thr Asp Tyr Tyr Arg Ile Pro Ala		
305	310	315
Ile Ala Gln Ala Pro Asn Gly Trp Ile Leu Ala Ala Trp Asp Leu Arg		
325	330	335
Pro Ser Ser Ala Ala Asp Ala Pro Asn Pro Asn Ser Ile Val Gln Arg		
340	345	350
Ile Ser Lys Asp Gly Gly Lys Ser Trp Glu Thr Leu Ala Tyr Val Ala		
355	360	365
Gln Gly Arg Ser Ala Thr Asn Lys Tyr Gly Tyr Ser Asp Pro Ser Tyr		
370	375	380
Val Val Asp Glu Glu Ala Gly Lys Ile Phe Leu Phe Cys Val Lys Ser		
385	390	395
Tyr Asp Gln Gly Tyr Phe Gly Ser Val Leu Gly Val Glu Asp Ala Arg		
405	410	415
Asn Val Leu Gln Ala Val Val Met Glu Ser Asp Asp Asn Gly Ala Thr		
420	425	430
Trp Ser Glu Pro Arg Asn Ile Thr Lys Asp Ile Thr Lys Gly His Glu		
435	440	445
Asp Glu Trp Lys Ser Arg Phe Ala Ser Ser Gly His Gly Ile Gln Leu		
450	455	460
Lys Tyr Gly Gln Tyr Lys Gly Arg Leu Ile Gln Gln Tyr Ala Val Arg		
465	470	475
Thr Thr Ser Asn Thr Asn Ile Ala Val Ser Val Tyr Ser Asp Asp His		
485	490	495
Gly Lys Thr Trp Lys Ala Gly Asn Pro Val Thr Glu Val Asn Met Asp		
500	505	510
Glu Asn Lys Val Val Glu Leu Ser Asp Gly Arg Val Met Leu Asn Ser		
515	520	525
Arg Pro Gly Ala Ala Gly Tyr Arg Arg Val Ala Ile Ser Glu Asp Gly		
530	535	540
Gly Val Asn Tyr Gly Pro Ile Lys Ser Glu Thr Gln Leu Pro Asp Pro		
545	550	555
Asn Asn Asn Ala Gln Ile Thr Arg Ala Phe Pro Asn Ala Pro Glu Gly		
565	570	575
Ser Ala Lys Ala Lys Val Leu Leu Tyr Ser Ala Pro Arg Ala Ser Asn		
580	585	590
Glu Gly Arg Ala Asn Gly Val Val Arg Val Ser Phe Asp Asp Gly Thr		
595	600	605

-continued

Thr Trp Ser Ala Gly Lys Leu Phe Lys Glu Gly Ser Met Ala Tyr Ser
 610 615 620
 Val Ile Thr Ala Leu Asn Asp Ala Ala Gly Gly Tyr Gly Leu Leu
 625 630 635 640
 Tyr Glu Gly Glu Ser Ile Thr Tyr Thr Arg Ala Ser Met Glu Trp Leu
 645 650 655
 Gly Tyr Leu Thr Ala Thr Ala Ser Gly Thr Ala Thr Val Lys Glu Gly
 660 665 670
 Glu Gly Thr Leu Thr Ala Pro Val Thr Val Thr Asn Asp Gly Leu Thr
 675 680 685
 Asp Tyr Thr Asn Val Asn Val Thr Pro Thr Gly Leu Pro Ser Gly Trp
 690 695 700
 Ser Ala Glu Ala Val Asn Val Gly Asn Leu Ala Ala Gly Gln Ser Ala
 705 710 715 720
 Thr Val Asn Val Pro Val Thr Val Pro Ala Ala Ala Val Ser Gly Thr
 725 730 735
 Val Ala Lys Ala Thr Met Lys Ile Thr Gly Lys Tyr Ala Gln Ser Glu
 740 745 750
 Asp Thr Leu His Ser Phe Ala Glu Gly Glu Leu Ala Val Thr Val Thr
 755 760 765
 Asp Pro Asp Pro Ala Ala Lys Arg Leu Lys Leu Thr Ile Glu Arg Thr
 770 775 780
 Asp Asp Asn Gly Asp Pro Val Lys Val Gly Asp Thr Leu Thr Tyr Arg
 785 790 795 800
 Ile Thr Tyr Glu Asn Val Gly Thr Gln Ser Phe Ala Val Tyr Pro Arg
 805 810 815
 Glu Ser Asn Leu Asp Gly Val Thr Thr Pro Gln Ser Ala Ser Asn Pro
 820 825 830
 Ala Pro Val Cys Arg Trp Ser Arg Leu Ala Pro Gly Thr Thr Gly Ala
 835 840 845
 Cys Val Ser Gly Asn Gly Lys Gln Leu Ala Tyr His Thr Val Thr Glu
 850 855 860
 Ala Asp Ala Thr Ala Gly Ser Phe Thr Pro Ser Ala Thr Ile Asp Ala
 865 870 875 880
 Thr Ala Asp Ala Ser Gly Glu Met Val Leu Glu Ser Val Ser Ile Thr
 885 890 895
 Gly Asp Pro Val Thr Val Ser Gln Pro Val Glu Leu Pro Ala Asp Ile
 900 905 910
 Ala Ala Trp Lys Thr Arg Asn Glu Ala Leu Asp Asp Trp Gln Ala Leu
 915 920 925
 Ser Glu Lys Leu Ala Lys Thr Asp Arg Ile Asn Trp Leu Phe Thr Gly
 930 935 940
 Asp Ser Ile Thr His Gly Val Gln Phe Thr Arg Gly Tyr Arg Thr Tyr
 945 950 955 960
 Ser Glu Leu Phe Ala Asn His Leu Asp Thr Ala Ser Val Arg Gly Val
 965 970 975
 Ser Arg Ala Asn Asp Val Val Met Asn Thr Gly Ile Ser Ser Ala Asp
 980 985 990
 Ala Ser Trp Pro Leu Lys Asp Gly Ala Phe Glu Lys Trp Val Ser Asp
 995 1000 1005
 Lys His Pro Asp Val Val Phe Leu Thr Phe Gly Met Asn Asp Gly Arg
 1010 1015 1020

-continued

Thr Gly Gln Ala Phe Thr Val Asp Gln Tyr Thr Ala Asn Leu Ser Thr
 1025 1030 1035 1040
 Leu Ile Asp Lys Ile Arg Asp Ile Gly Ala Ile Pro Val Leu Gln Thr
 1045 1050 1055
 Gln Asn Tyr Thr Thr Asn Thr Phe Asn Ala Asn Leu Asp Thr Tyr
 1060 1065 1070
 Phe Asp Ala Glu Arg Arg Leu Ala Leu Asp Lys Asn Val Leu Leu Val
 1075 1080 1085
 Asp Phe Asn Lys Gln Trp Leu Ala Leu Gly Gly Asn Arg Glu Ser
 1090 1095 1100
 Gly Thr Tyr Met Gly Ala Gly Asn Asp Ile His Pro Gly Glu Asn Gly
 1105 1110 1115 1120
 His Ile Glu Trp Ala Lys Phe Thr Leu Gly Ala Leu Asn Met Ile Ala
 1125 1130 1135
 Asn Asp Asp Pro Leu Ala Arg Trp Ser Ser Ser Asp Thr Thr Leu Asp
 1140 1145 1150
 Lys Pro Thr Val Thr Val Asp Ala Asp Gly Asn Gly Leu Lys Gly Ser
 1155 1160 1165
 Asp Gly Leu Glu Pro Ala Pro Ala Ala Ala Lys Ser Val Gly Lys Phe
 1170 1175 1180
 Leu Ser Gly Ala Gln Tyr Val Asp Leu Gly Gly Asp Val Val Ser Ala
 1185 1190 1195 1200
 Val Ala Gly Lys Arg Glu Ser Asn Val Thr Ile Arg Phe Arg Ala Ser
 1205 1210 1215
 Ala Thr Gly Gln Pro Gln Thr Leu Phe Ser Leu Gly Asp Ser Asp Ser
 1220 1225 1230
 Ala Thr Arg Ala Thr Val Arg Leu Ser Ala Thr Gly Leu Val Gln Phe
 1235 1240 1245
 Leu Asn Ser Gly Asn Thr Gly Asp Phe Tyr Thr Val Gly Thr Asn Asp
 1250 1255 1260
 Leu Ala Asp Gly Ala Trp His Thr Val Ser Val Asn Phe Val Ala Asn
 1265 1270 1275 1280
 Gly Phe Thr Ile Tyr Val Asp Gly Ala Ala Met Arg Ala Ile Ser Gly
 1285 1290 1295
 Gly Thr Gly Thr Gln Leu Asn Val Pro Gly Ala Ile Thr Val Asn Thr
 1300 1305 1310
 Ala Thr Ala Gly Ala Ile Arg Gly Ala Asp Ser Ala Gly Gly Ala Gln
 1315 1320 1325
 Gln Leu Thr Gly Ile Val Asp Tyr Val Ala Ala Trp Ser Arg Thr Leu
 1330 1335 1340
 Thr Gly Ala Glu Ala Lys Arg Ile Ser Ala Glu Thr Ser Ala Val Ala
 1345 1350 1355 1360
 Val Thr Lys Val Asp Ala Ala Val Asn Ala Leu Gln Pro Ile Ile Ser
 1365 1370 1375
 Asp Thr Gly Ala Arg Lys Asn Ile Val Phe Val Gly Gly Glu Thr Ile
 1380 1385 1390
 Glu Gly Gly Tyr Thr Asp His Leu Ile Ala Lys Asn Ile Val Gln Leu
 1395 1400 1405
 Leu Asp Glu Arg Val Arg Trp Glu Tyr Val Thr Gly Leu Ser Ala Thr
 1410 1415 1420
 Asp Arg Glu Arg Gln Arg Ala Lys Phe Phe Val Ala Ala Gly Gln Gly
 1425 1430 1435 1440
 Gly Leu Thr Ala Lys Gln Met Asp Glu Asp Tyr Ala Ala Met Val Gly

-continued

1445	1450	1455
Glu Tyr Ser Pro Asp Ile Leu Phe Leu Ala Pro Asp Leu Tyr Asp Ala		
1460	1465	1470
Asp Gly Asn Leu Ala Glu Ser Asp Ala Ala Ala Phe Ala Gly His Ile		
1475	1480	1485
Arg Ser Val Ala Ala Lys Ala Lys Glu Ala Gly Ala Lys Val Val Leu		
1490	1495	1500
Val Thr Pro Val Thr Val Arg Gly Gly Glu Asp Glu Tyr Ala Gly Ala		
1505	1510	1515
Met Arg Thr Val Ala Lys Glu Asp Asp Leu Pro Leu Ile Asp Ala Gln		
1525	1530	1535
Ala Trp Ile Gly Lys Val Val Ala Ala Asp Ala Ser Val Lys Thr Ala		
1540	1545	1550
Trp Phe Asn Lys Ala Gly Gln Leu Asn Tyr Ala Gly His Leu Gly Tyr		
1555	1560	1565
Ala Arg Phe Met Met Arg Ser Leu Asp Leu Tyr Pro Ser Asn Val Ser		
1570	1575	1580
Gly Ser Arg Ile Ala Ser Leu Pro Tyr Asp Thr Ala Asn Val Thr Leu		
1585	1590	1595
Val Gly Ala Ser Glu Asn Gly Gly Glu Leu Pro Val Gly Arg Val Glu		
1605	1610	1615
Gly Thr Asp Arg Ala His Ile Asp Thr Met Gln Ile Gly Ala Ala Ala		
1620	1625	1630
Ser Leu Val Val Ala Asp Ser Tyr Ala Val Tyr Glu Ile Gly Glu Asp		
1635	1640	1645
Gly Gly Arg Thr Leu Val Ala Asp Gly Leu Lys Pro Ala Asp Val Leu		
1650	1655	1660
Ala Asp Gly Ile Asp Val Thr Val Asn Asp Thr Ala Ala His Arg Tyr		
1665	1670	1675
Glu Val Val Gly Ser Ala Asn Val Pro Glu Gly Ala Asp Ala Val Thr		
1685	1690	1695
Val Thr Tyr Thr Ala Thr Leu Ala Ala Val Glu Glu Pro Glu Pro Gly		
1700	1705	1710
Pro Asp Pro Asp Pro Thr Pro Asp Pro Ser Glu Lys Pro Asp Gly Asp		
1715	1720	1725
Gly Thr Gly Asp Gly Thr Gly Ala Gly Thr Gly Asp Val Gln Lys Pro		
1730	1735	1740
Thr Pro Asp Ala Val Ala Lys Thr Gly Ala Asp Val Phe Gly Leu Leu		
1745	1750	1755
Ala Ala Val Ala Val Leu Leu Ala Ala Gly Ser Val Thr Leu Ser Leu		
1765	1770	1775
Arg Arg Arg Ala Asn Arg		
1780		

<210> SEQ ID NO 9
<211> LENGTH: 835
<212> TYPE: PRT
<213> ORGANISM: Bifidobacterium bifidum
<220> FEATURE:
<221> NAME/KEY: source
<222> LOCATION: 1..835
<223> OTHER INFORMATION: /mol_type="protein"
/organism="Bifidobacterium bifidum"

<400> SEQUENCE: 9

Met Val Arg Ser Thr Lys Pro Ser Leu Leu Arg Arg Phe Gly Ala Leu

-continued

1	5	10	15
Val Ala Ala Ala Ala Met Leu Val Val Leu Pro Ala Gly Val Ser Thr			
20	25	30	
Ala Ser Ala Ala Ser Asp Asp Ala Asp Met Leu Thr Val Thr Met Thr			
35	40	45	
Arg Thr Asp Ala Leu Gly Asp Glu Val Tyr Val Gly Asp Thr Leu Thr			
50	55	60	
Tyr Ser Phe Thr Asn Thr Asn Asn Thr Ser Ser Ala Phe Thr Ala Phe			
65	70	75	80
Pro Ala Glu Ser Asn Leu Ser Gly Val Leu Thr Thr Gly Thr Pro Asn			
85	90	95	
Cys Arg Tyr Glu Asn Leu Ala Gly Gly Ala Ser Tyr Pro Cys Ser Thr			
100	105	110	
Ala Ser His Thr Ile Thr Ala Asp Asp Leu Thr Ala Gly Ser Phe Thr			
115	120	125	
Pro Arg Thr Val Trp Lys Ala Thr Ser Asp Arg Gly Gly Thr Gln Val			
130	135	140	
Leu Gln Asp Asn Ile Val Ser Thr Gly Asp Thr Val Thr Val Lys Glu			
145	150	155	160
Gly Lys Arg Pro Asp Pro Ala Thr Ile Pro Thr Asp Arg Ala Asp Gly			
165	170	175	
Glu Ala Val Arg Leu Ala Thr Ala Arg Gln Asn Leu Gly Thr Glu Cys			
180	185	190	
Tyr Arg Ile Pro Ala Leu Ala Glu Ala Pro Asn Gly Trp Ile Leu Ala			
195	200	205	
Ala Phe Asp Gln Arg Pro Asn Thr Ala Met Ala Asn Gly Ser Gly Val			
210	215	220	
Lys Cys Trp Asp Ala Pro Gln Pro Asn Ser Ile Val Gln Arg Ile Ser			
225	230	235	240
Lys Asp Gly Gly Lys Ser Trp Thr Pro Ile Gln Tyr Val Ala Gln Gly			
245	250	255	
Lys Asn Ala Pro Glu Arg Tyr Gly Tyr Ser Asp Pro Ser Tyr Val Val			
260	265	270	
Asp Lys Glu Thr Gly Glu Ile Phe Leu Phe Phe Val His Ser Tyr Asn			
275	280	285	
Lys Gly Phe Ala Asp Ser Gln Leu Gly Val Asp Glu Ser Asn Arg Asn			
290	295	300	
Val Leu His Ala Val Val Val Ser Ser Lys Asp Asn Gly Glu Thr Trp			
305	310	315	320
Ser Lys Pro Arg Asp Ile Thr Ala Asp Ile Thr Lys Gly Tyr Glu Asn			
325	330	335	
Glu Trp Lys Ser Arg Phe Ala Thr Ser Gly Ala Gly Ile Gln Leu Lys			
340	345	350	
Tyr Gly Lys Tyr Lys Gly Arg Leu Ile Gln Gln Tyr Ala Val Gly Arg			
355	360	365	
Thr Thr Gly Ser Asn Ala Ala Val Ser Val Tyr Ser Asp Asp His Gly			
370	375	380	
Lys Thr Trp Gln Ala Gly Asn Pro Val Thr Gly Met Leu Met Asp Glu			
385	390	395	400
Asn Lys Val Val Glu Leu Ser Asp Gly Arg Val Met Leu Asn Ser Arg			
405	410	415	
Pro Gly Ala Ala Gly Tyr Arg Arg Val Ala Ile Ser Glu Asp Gly Gly			
420	425	430	

-continued

Val Asn Tyr Gly Thr Val Lys Asn Glu Thr Gln Leu Pro Asp Pro Asn
 435 440 445

 Asn Asn Ala His Ile Thr Arg Ala Phe Pro Asn Ala Pro Glu Gly Ser
 450 455 460

 Ala Lys Ala Lys Val Leu Leu Tyr Ser Ser Pro Arg Ala Asn Asn Glu
 465 470 475 480

 Gly Arg Ala Asn Gly Val Val Arg Ile Ser Leu Asp Asp Gly Thr Thr
 485 490 495

 Trp Ser Ser Gly Lys Leu Tyr Lys Glu Gly Ser Met Ala Tyr Ser Val
 500 505 510

 Ile Thr Ala Leu Ser Asp Ala Ala Gly Gly Tyr Gly Leu Leu Tyr
 515 520 525

 Glu Gly Ala Trp Val Thr Gly Gly Ile Asp Ser His Asp Ile Met
 530 535 540

 Tyr Thr His Ile Ser Met Asp Trp Leu Gly Tyr Leu Ser Ala Thr Ala
 545 550 555 560

 Asp Asp Val Thr Ala Ser Val Glu Gly Ala Ser Thr Val Asp Val
 565 570 575

 Thr Val Pro Val Ser Asn Val Gly Ser Val Asp Tyr Thr Gly Val Thr
 580 585 590

 Val Thr Pro Ala Asp Leu Pro Thr Gly Trp Ser Ala Ser Pro Val Asn
 595 600 605

 Val Gly Ala Leu Ala Ser Gly Thr Ser Lys Asp Val Thr Val Thr Val
 610 615 620

 Asn Val Pro Ala Thr Ala Lys Lys Asp Asp Val Ala Lys Ile Val Leu
 625 630 635 640

 Arg Val Thr Gly Thr Ser Ala Ala Asn Ala Asn Ala Thr Thr Gly Phe
 645 650 655

 Asp Gly Ser Ile Thr Val Asn Val Thr Glu Lys Ser Glu Pro Asp Pro
 660 665 670

 Glu Pro Glu Pro Thr Ile Thr Gly Val Ser Ala Val Thr Ser Gln Ala
 675 680 685

 Gly Val Lys Val Gly Asp Val Phe Asp Ala Ser Lys Val Ser Val Thr
 690 695 700

 Ala Ala Met Ser Asp Gly Ser Ser Lys Ala Leu Ala Ala Gly Glu Tyr
 705 710 715 720

 Ser Leu Ser Ala Val Asp Ala Asp Gly Lys Ala Val Asp Leu Ala Glu
 725 730 735

 Pro Phe Ala Ala Ala Gly Val Val Thr Val Thr Val Ser Val Pro Val
 740 745 750

 Glu Gly Ala Gly Pro Leu Thr Ala Ser Phe Thr Ile Asp Val Ala Glu
 755 760 765

 Lys Ser Val Asp Pro Glu Pro Lys Pro Glu Pro Glu Pro Lys Pro Glu
 770 775 780

 Pro Glu Lys Pro Ala Gly Pro Lys Val Asp Val Pro Thr Glu Gln Pro
 785 790 795 800

 Gly Leu Ser Lys Thr Gly Ala Ser Thr Ala Gly Met Ser Ile Val Phe
 805 810 815

 Val Leu Leu Ala Leu Ser Gly Ile Ala Ala Leu Ser Leu Arg Arg Arg
 820 825 830

 Ser Val His
 835

-continued

```

<210> SEQ ID NO 10
<211> LENGTH: 1060
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi
<220> FEATURE:
<221> NAME/KEY: source
<222> LOCATION: 1..1060
<223> OTHER INFORMATION: /mol_type="protein"
/organism="Trypanosoma cruzi"

<400> SEQUENCE: 10

Met Gly Lys Thr Val Val Gly Ala Ser Arg Met Phe Trp Leu Met Phe
1           5          10          15

Phe Val Pro Leu Leu Leu Ala Leu Cys Pro Ser Glu Pro Ala His Ala
20          25          30

Leu Ala Pro Gly Ser Ser Arg Val Glu Leu Phe Lys Arg Gln Ser Ser
35          40          45

Lys Val Pro Phe Glu Lys Gly Lys Val Thr Glu Arg Val Val His
50          55          60

Ser Phe Arg Leu Pro Ala Leu Val Asn Val Asp Gly Val Met Val Ala
65          70          75          80

Ile Ala Asp Ala Arg Tyr Glu Thr Ser Asn Asp Asn Ser Leu Ile Asp
85          90          95

Thr Val Ala Lys Tyr Ser Val Asp Asp Gly Glu Thr Trp Glu Thr Gln
100         105         110

Ile Ala Ile Lys Asn Ser Arg Ala Ser Ser Val Ser Arg Val Val Asp
115         120         125

Pro Thr Val Ile Val Lys Gly Asn Lys Leu Tyr Val Leu Val Gly Ser
130         135         140

Tyr Asn Ser Ser Arg Ser Tyr Trp Thr Ser His Gly Asp Ala Arg Asp
145         150         155         160

Trp Asp Ile Leu Leu Ala Val Gly Glu Val Thr Lys Ser Thr Ala Gly
165         170         175

Gly Lys Ile Thr Ala Ser Ile Lys Trp Gly Ser Pro Val Ser Leu Lys
180         185         190

Glu Phe Phe Pro Ala Glu Met Glu Gly Met His Thr Asn Gln Phe Leu
195         200         205

Gly Gly Ala Gly Val Ala Ile Val Ala Ser Asn Gly Asn Leu Val Tyr
210         215         220

Pro Val Gln Val Thr Asn Lys Lys Gln Val Phe Ser Lys Ile Phe
225         230         235         240

Tyr Ser Glu Asp Glu Gly Lys Thr Trp Lys Phe Gly Glu Gly Arg Ser
245         250         255

Asp Phe Gly Cys Ser Glu Pro Val Ala Leu Glu Trp Glu Gly Lys Leu
260         265         270

Ile Ile Asn Thr Arg Val Asp Tyr Arg Arg Arg Leu Val Tyr Glu Ser
275         280         285

Ser Asp Met Gly Asn Ser Trp Val Glu Ala Val Gly Thr Leu Ser Arg
290         295         300

Val Trp Gly Pro Ser Pro Lys Ser Asn Gln Pro Gly Ser Gln Ser Ser
305         310         315         320

Phe Thr Ala Val Thr Ile Glu Gly Met Arg Val Met Leu Phe Thr His
325         330         335

Pro Leu Asn Phe Lys Gly Arg Trp Leu Arg Asp Arg Leu Asn Leu Trp
340         345         350

```

-continued

Leu Thr Asp Asn Gln Arg Ile Tyr Asn Val Gly Gln Val Ser Ile Gly
 355 360 365
 Asp Glu Asn Ser Ala Tyr Ser Ser Val Leu Tyr Lys Asp Asp Lys Leu
 370 375 380
 Tyr Cys Leu His Glu Ile Asn Ser Asn Glu Val Tyr Ser Leu Val Phe
 385 390 395 400
 Ala Arg Leu Val Gly Glu Leu Arg Ile Ile Lys Ser Val Leu Gln Ser
 405 410 415
 Trp Lys Asn Trp Asp Ser His Leu Ser Ser Ile Cys Thr Pro Ala Asp
 420 425 430
 Pro Ala Ala Ser Ser Ser Glu Arg Gly Cys Gly Pro Ala Val Thr Thr
 435 440 445
 Val Gly Leu Val Gly Phe Leu Ser His Ser Ala Thr Lys Thr Glu Trp
 450 455 460
 Glu Asp Ala Tyr Arg Cys Val Asn Ala Ser Thr Ala Asn Ala Glu Arg
 465 470 475 480
 Val Pro Asn Gly Leu Lys Phe Ala Gly Val Gly Gly Ala Leu Trp
 485 490 495
 Pro Val Ser Gln Gln Gly Gln Asn Gln Arg Tyr His Phe Ala Asn His
 500 505 510
 Ala Phe Thr Leu Val Ala Ser Val Thr Ile His Glu Val Pro Ser Val
 515 520 525
 Ala Ser Pro Leu Leu Gly Ala Ser Leu Asp Ser Ser Gly Gly Lys Lys
 530 535 540
 Leu Leu Gly Leu Ser Tyr Asp Glu Lys His Gln Trp Gln Pro Ile Tyr
 545 550 555 560
 Gly Ser Thr Pro Val Thr Pro Thr Gly Ser Trp Glu Met Gly Lys Arg
 565 570 575
 Tyr His Val Val Leu Thr Met Ala Asn Lys Ile Gly Ser Val Tyr Ile
 580 585 590
 Asp Gly Glu Pro Leu Glu Gly Ser Gly Gln Thr Val Val Pro Asp Gly
 595 600 605
 Arg Thr Pro Asp Ile Ser His Phe Tyr Val Gly Gly Tyr Gly Arg Ser
 610 615 620
 Asp Met Pro Thr Ile Ser His Val Thr Val Asn Asn Val Leu Leu Tyr
 625 630 635 640
 Asn Arg Gln Leu Asn Ala Glu Glu Ile Arg Thr Leu Phe Leu Ser Gln
 645 650 655
 Asp Leu Ile Gly Thr Glu Ala His Met Gly Ser Ser Ser Gly Ser Ser
 660 665 670
 Ala His Ser Thr Pro Ser Thr Pro Ala Asp Asn Gly Ala His Ser Thr
 675 680 685
 Pro Ser Thr Pro Ala Asp Ser Ser Ala His Ser Thr Pro Ser Thr Pro
 690 695 700
 Ala Asp Ser Ser Ala His Ser Thr Pro Ser Ala Pro Gly Asp Asn Gly
 705 710 715 720
 Ala His Ser Thr Pro Ser Thr Pro Gly Asp Ser Ser Ala His Ser Thr
 725 730 735
 Pro Ser Thr Pro Ala Asp Asn Gly Ala His Ser Thr Pro Ser Ala Pro
 740 745 750
 Ala Asp Ser Asn Ala His Ser Thr Pro Ser Thr Pro Ala Asp Asn Gly
 755 760 765
 Ala His Ser Thr Pro Ser Thr Pro Ala Asp Asn Gly Ala His Ser Thr

US 9,102,966 B2

93**94**

-continued

770	775	780
Pro Ser Thr Pro Gly Asp Asn Gly Ala His Ser	Thr Pro Ser Thr Pro	
785 790 795 800		
Gly Asp Ser Ser Ala His Ser Thr Pro Ser Thr Pro	Ala Asp Asn Gly	
805 810 815		
Ala His Ser Thr Pro Ser Ala Pro Ala Asp Ser Asn	Ala His Ser Thr	
820 825 830		
Pro Ser Thr Pro Gly Asp Asn Gly Ala His Ser Thr	Pro Ser Ala Pro	
835 840 845		
Ala Asp Ser Asn Ala His Ser Thr Pro Ser Thr Pro	Ala Asp Ser Ser	
850 855 860		
Ala His Ser Thr Pro Ser Ala Pro Gly Asp Asn Gly	Ala His Ser Thr	
865 870 875 880		
Pro Ser Ala Pro Ala Asp Ser Ser Ala His Ser Thr	Pro Ser Ala Pro	
885 890 895		
Gly Asp Asn Gly Ala His Ser Thr Pro Ser Ala Pro	Ala Asp Asn Gly	
900 905 910		
Ala His Ser Thr Pro Ser Ala Pro Gly Asp Ser Asn	Ala His Ser Thr	
915 920 925		
Pro Ser Thr Pro Ala Asp Ser Ser Ala His Ser Thr	Pro Ser Thr Pro	
930 935 940		
Ala Asp Ser Ser Ala His Ser Thr Pro Ser Ala Pro	Gly Asp Asn Gly	
945 950 955 960		
Ala His Ser Thr Pro Ser Ala Pro Ala Asp Ser Ser	Ala His Ser Thr	
965 970 975		
Pro Ser Ile Pro Gly Asp Ser Ser Ala His Ser Thr	Pro Ser Ala Pro	
980 985 990		
Ala Asp Ser Ser Ala His Ser Thr Pro Ser Ala Pro	Gly Asp Asn Gly	
995 1000 1005		
Ala His Ser Thr Pro Ser Thr Pro Ala Asp Asn Gly	Ala Asn Gly Thr	
1010 1015 1020		
Val Leu Ile Leu His Asp Gly Ala Ala Phe Ser	Ala Phe Ser Gly Gly	
1025 1030 1035 1040		
Gly Leu Leu Leu Cys Ala Gly Ala Leu Leu Leu His	Val Phe Val Met	
1045 1050 1055		
Ala Val Phe Phe		
1060		
<210> SEQ ID NO 11		
<211> LENGTH: 642		
<212> TYPE: PRT		
<213> ORGANISM: Trypanosoma cruzi		
<220> FEATURE:		
<221> NAME/KEY: source		
<222> LOCATION: 1..642		
<223> OTHER INFORMATION: /mol_type="protein"		
/organism="Trypanosoma cruzi"		
<400> SEQUENCE: 11		
Met Leu Ala Pro Gly Ser Ser Arg Val Glu Leu Phe Lys Arg Gln Ser		
1	5	10 15
Ser Lys Val Pro Phe Glu Lys Asp Gly Lys Val Thr Glu Arg Val Val		
20	25	30
His Ser Phe Arg Leu Pro Ala Leu Val Asn Val Asp Gly Val Met Val		
35	40	45
Ala Ile Ala Asp Ala Arg Tyr Glu Thr Ser Asn Asn Ser Leu Ile		

US 9,102,966 B2

95**96**

-continued

50	55	60
Asp Thr Val Ala Lys Tyr Ser Val Asp Asp Gly Glu Thr Trp Glu Thr		
65	70	75
80		
Gln Ile Ala Ile Lys Asn Ser Arg Ala Ser Ser Val Ser Arg Val Val		
85	90	95
Asp Pro Thr Val Ile Val Lys Gly Asn Lys Leu Tyr Val Leu Val Gly		
100	105	110
Ser Tyr Asn Ser Ser Arg Ser Tyr Trp Thr Ser His Gly Asp Ala Arg		
115	120	125
Asp Trp Asp Ile Leu Leu Ala Val Gly Glu Val Thr Lys Ser Thr Ala		
130	135	140
Gly Gly Lys Ile Thr Ala Ser Ile Lys Trp Gly Ser Pro Val Ser Leu		
145	150	155
160		
Lys Glu Phe Phe Pro Ala Glu Met Glu Gly Met His Thr Asn Gln Phe		
165	170	175
Leu Gly Gly Ala Gly Val Ala Ile Val Ala Ser Asn Gly Asn Leu Val		
180	185	190
Tyr Pro Val Gln Val Thr Asn Lys Lys Gln Val Phe Ser Lys Ile		
195	200	205
Phe Tyr Ser Glu Asp Glu Gly Lys Thr Trp Lys Phe Gly Lys Gly Arg		
210	215	220
Ser Ala Phe Gly Cys Ser Glu Pro Val Ala Leu Glu Trp Glu Gly Lys		
225	230	235
240		
Leu Ile Ile Asn Thr Arg Val Asp Tyr Arg Arg Arg Leu Val Tyr Glu		
245	250	255
Ser Ser Asp Met Gly Asn Ser Trp Leu Glu Ala Val Gly Thr Leu Ser		
260	265	270
Arg Val Trp Gly Pro Ser Pro Lys Ser Asn Gln Pro Gly Ser Gln Ser		
275	280	285
Ser Phe Thr Ala Val Thr Ile Glu Gly Met Arg Val Met Leu Phe Thr		
290	295	300
His Pro Leu Asn Phe Lys Gly Arg Trp Leu Arg Asp Arg Leu Asn Leu		
305	310	315
320		
Trp Leu Thr Asp Asn Gln Arg Ile Tyr Asn Val Gly Gln Val Ser Ile		
325	330	335
Gly Asp Glu Asn Ser Ala Tyr Ser Ser Val Leu Tyr Lys Asp Asp Lys		
340	345	350
Leu Tyr Cys Leu His Glu Ile Asn Ser Asn Glu Val Tyr Ser Leu Val		
355	360	365
Phe Ala Arg Leu Val Gly Glu Leu Arg Ile Ile Lys Ser Val Leu Gln		
370	375	380
Ser Trp Lys Asn Trp Asp Ser His Leu Ser Ser Ile Cys Thr Pro Ala		
385	390	395
400		
Asp Pro Ala Ala Ser Ser Ser Glu Arg Gly Cys Gly Pro Ala Val Thr		
405	410	415
Thr Val Gly Leu Val Gly Phe Leu Ser His Ser Ala Thr Lys Thr Glu		
420	425	430
Trp Glu Asp Ala Tyr Arg Cys Val Asn Ala Ser Thr Ala Asn Ala Glu		
435	440	445
Arg Val Pro Asn Gly Leu Lys Phe Ala Gly Val Gly Gly Ala Leu		
450	455	460
Trp Pro Val Ser Gln Gln Gly Gln Asn Gln Arg Tyr Arg Phe Ala Asn		
465	470	475
480		

-continued

His Ala Phe Thr Val Val Ala Ser Val Thr Ile His Glu Val Pro Ser
 485 490 495

Val Ala Ser Pro Leu Leu Gly Ala Ser Leu Asp Ser Ser Gly Gly Lys
 500 505 510

Lys Leu Leu Gly Leu Ser Tyr Asp Glu Arg His Gln Trp Gln Pro Ile
 515 520 525

Tyr Gly Ser Thr Pro Val Thr Pro Thr Gly Ser Trp Glu Met Gly Lys
 530 535 540

Arg Tyr His Val Val Leu Thr Met Ala Asn Lys Ile Gly Ser Glu Tyr
 545 550 555 560

Ile Asp Gly Glu Pro Leu Glu Gly Ser Gly Gln Thr Val Val Pro Asp
 565 570 575

Glu Arg Thr Pro Asp Ile Ser His Phe Tyr Val Gly Gly Tyr Lys Arg
 580 585 590

Ser Asp Met Pro Thr Ile Ser His Val Thr Val Asn Asn Val Leu Leu
 595 600 605

Tyr Asn Arg Gln Leu Asn Ala Glu Ile Arg Thr Leu Phe Leu Ser
 610 615 620

Gln Asp Leu Ile Gly Thr Glu Ala His Met Asp Ser Ser Ser Asp Thr
 625 630 635 640

Ser Ala

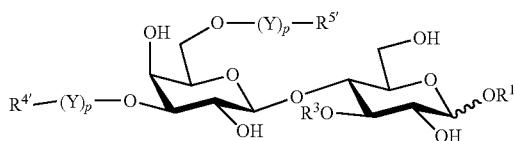
30

The invention claimed is:

1. Method for the synthesis of compounds of human milk oligosaccharides of Formula 1-3D and salts thereof

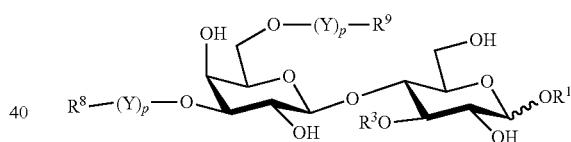
oligosaccharide, glycolipid, glycoprotein or glycopeptide, cyclic or acyclic aliphatic group, or aryl residue, and SA is an α -sialyl moiety, is reacted with a sialyl acceptor of Formula 2D and salts thereof

1-3D 35

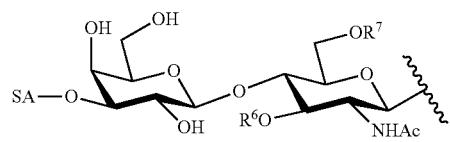


30

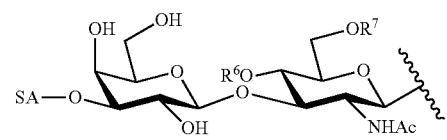
2D



wherein Y is independently an N-acetyl-lactosaminyl group optionally substituted with a sialyl and/or fucosyl residue, integer p is independently 0, 1 or 2, R⁴ is selected from Formulae 3-3 or 4-3,



3-3

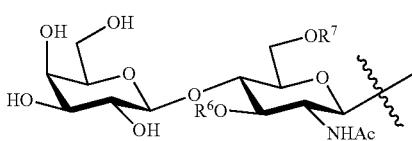


4-3

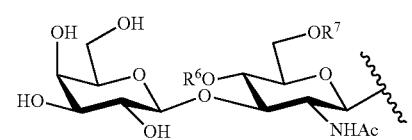
wherein R⁶ is H or fucosyl residue, R⁷H or α -sialyl moiety, SA is α -sialyl moiety, and R^{5'} is selected from the group consisting of H, α -sialyl moiety, a group of Formula 3-3, and a group of Formula 4-3; wherein R¹ is a protecting group that is removable by hydrogenolysis, in which a sialyl donor of formula SA-OR² and salts thereof, wherein R² is a mono-, di- or

wherein R¹ is as defined above and is a group removable by hydrogenolysis, in which a sialyl donor of formula SA-OR³ is H or fucosyl unit, Y is independently an N-acetyl-lactosaminyl group optionally substituted with a sialyl and/or fucosyl residue, p is an integer independently selected from 0, 1 or 2, R⁸ is selected from Formulae 5 or 6,

50



5



6

wherein R⁶ is H or fucosyl residue, R⁷H or α -sialyl moiety, and R⁹ is selected from the group consisting of H, α -sialyl moiety, a group of Formula 5 and a group of Formula 6, under the catalysis of an enzyme having transsialidase activity.

2. The method according to claim 1, wherein the enzyme having transsialidase activity is selected from sialidases derived from *Bifidobacterium* species and transsialidases derived from *Trypanosoma cruzi*.

3. The method according to claim 1, wherein the enzyme having transsialidase activity is an engineered enzyme.

4. The method according to claim 1, wherein the sialyl donor is selected from the group consisting of 2-O-(p-nitrophenyl)- α -D-sialoside, 2-O-(4-methylumbelliferyl)- α -D-sialoside, fetuin and 3'-O-sialyl-lactose.

5. The method according to claim 1, wherein R₁ is selected from the group consisting of benzyl or 2-naphthylmethyl groups optionally substituted with at least one group selected from the group consisting of phenyl, alkyl or halogen.

6. Method for the synthesis of compounds selected from the group consisting of R¹-glycosides of Neu5Ac α 2-3Gal β 1-4(Fuc α 1-3)Glc (3-O-fucosyl-3'-O-(N-acetyl-neuramino-syl)-lactose), Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc (LST a), Neu5Ac α 2-3Gal β 1-GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc (FLST a), Neu5Ac α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc, Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Ac α 2-3Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Ac α 2-3Gal β 1-3(Neu5Ac α 2-6)GlcNAc β 1-3Gal β 1-4Glc (DSLNT), Neu5Ac α 2-3Gal β 1-3(Neu5Ac α 2-6)(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc (FDSSLNT I), Neu5Ac α 2-3Gal β 1-3(Neu5Ac α 2-6)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc (FDSSLNT II), Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Neu5Ac α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc and salts thereof

wherein R¹ is a protecting group that is removable by hydrogenolysis, in which a sialyl donor of formula SA-

OR² and salts thereof, wherein R² is a mono-, di- or oligosaccharide, glycolipid, glycoprotein or glycopeptide, cyclic or acyclic aliphatic group, or aryl residue, and SA is an α -sialyl moiety, is reacted with a sialyl acceptor selected from the group of R¹-glycosides of Gal β 1-4(Fuc α 1-3)Glc (3-O-fucosyllactose), Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc (LNT), Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc (LNNT), Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc (LNFP II), Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc (LNFP III), Gal β 1-3GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc (LNFP V), Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc (LNDFH II), Gal β 1-3(Neu5Ac α 2-6)GlcNAc β 1-3Gal β 1-4Glc (LSTb), Gal β 1-3(Neu5Ac α 2-6)(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc, Gal β 1-3(Neu5Ac α 2-6)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc, Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc (LNDFH III), or salts thereof, under the catalysis of an enzyme having transsialidase activity.

7. The method according to claim 6, wherein R₁ is selected from the group consisting of benzyl or 2-naphthylmethyl groups optionally substituted with at least one group selected from the group consisting of phenyl, alkyl or halogen.

8. The method according to claim 6, wherein the enzyme having transsialidase activity is selected from sialidases derived from *Bifidobacterium* species and transsialidases derived from *Trypanosoma cruzi*.

9. The method according to claim 6, wherein the enzyme having transsialidase activity is an engineered enzyme.

10. The method according to claim 6, wherein the sialyl donor is selected from the group consisting of 2-O-(p-nitrophenyl)- α -D-sialoside, 2-O-(4-methylumbelliferyl)- α -D-sialoside, fetuin and 3'-O-sialyl-lactose.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 9,102,966 B2
APPLICATION NO. : 13/809794
DATED : August 11, 2015
INVENTOR(S) : Andreas Schroven et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On Title Page, Item (73) Assignee
replace “KGS, Lyngby (DK)”
with --Kgs. Lyngby (DK)--.

On Title Page, Item (56), References Cited, Other Publications, Line 29
replace “055:1-17”
with --055:H7”--.

Signed and Sealed this
Fifth Day of April, 2016



Michelle K. Lee
Director of the United States Patent and Trademark Office